Online ISSN : 2249-4618
Print ISSN : 0975-5888
DOI : 10.17406/GJMRA

Global Journal

of Medical Research: F

Diseases

Cancer, Ophthalmology & Pediatric

Local Immune Response

Cancerous Disease Gene Therapy

Highlights

Helicobacter Pylori Infection

Duodenal Brush Border Enzymes

Discovering Thoughts, Inventing Future

VOLUME 16 ISSUE 3 VERSION 1.0

Global Journal of Medical Research: F
Diseases
Cancer, Ophthalmology & Pediatric

Global Journal of Medical Research: F
Diseases
Cancer, Ophthalmology & Pediatric

Volume 16 Issue 3 (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® Headquarters
945th Concord Streets,
Framingham Massachusetts Pin: 01701,
United States of America
USA Toll Free: +001-888-839-7392
USA Toll Free Fax: +001-888-839-7392

Offset Typesetting

Global Journals Incorporated
2nd, Lansdowne, Lansdowne Rd., Croydon-Surrey,
Pin: CR9 2ER, United Kingdom

Packaging & Continental Dispatching

Global Journals
E-3130 Sudama Nagar, Near Gopur Square,
Indore, M.P., Pin: 452009, 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: *investors@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)

Integrated Editorial Board
(Computer Science, Engineering, Medical, Management, Natural Science, Social Science)

Dr. Bart Lambrecht
Director of Research in Accounting and Finance Professor 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 Regina Ph.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 Finance Lancaster University Management School Ph.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 Neuroscience Northwestern 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 Issue

Global Journal of Medical Research: F
Diseases
Volume 16 Issue 3 Version 1.0 Year 2016
Type: Double Blind Peer Reviewed International Research Journal
Publisher: Global Journals Inc. (USA)
Online ISSN: 2249-4618 & Print ISSN: 0975-5888

The Sensitivity and Specificity of Clinical Examination of the Hemodialysis Arterial-Venous Fistula (AVF) as Compared to Angiography

By Awad Magbri, Ji-Yank Sophie Lee, Eussera El-Magbri, Mariam El-Magbri & Taha El-Magbri

Dialysis Access Center of Pittsburgh, United States

Abstract- *Background and Objectives:* Physical examination of the hemodialysis arterial-venous fistula (AVF) is convenient and inexpensive, and can often detect common problems associated with hemodialysis access. Routine systematic physical examination of the fistula by the dialysis staff with each treatment may allow early detection of problems that are commonly associated with mature fistula. This avoiding missed treatments and emergent situations. Dialysis access stenosis is the most common cause of access dysfunction. Physical examination is an important method in the assessment of stenotic lesions. The purpose of this study is to evaluate the two simple maneuvers in physical examination of the AVF (pulse augmentation and pressure assessment inside the fistula and collapsibility of the fistula on arm elevation) and compare them with the gold standard angiography.

Keywords: *AVF (arteriovenous fistula), angiography, augmentation of the AVF, collapsibility of the AVF, interventional nephrologist, stenosis of the outflow tract of the fistula.*

GJMR-F Classification : *NLMC Code: WL141.5.C4*

Strictly as per the compliance and regulations of:

The Sensitivity and Specificity of Clinical Examination of the Hemodialysis Arterial-Venous Fistula (AVF) as Compared to Angiography

Awad Magbri [α], Ji-Yank Sophie Lee [σ], Eussera El-Magbri [ρ], Mariam El-Magbri [ω] & Taha El-Magbri [¥]

Abstract- ***Background and Objectives:*** Physical examination of the hemodialysis arterial-venous fistula (AVF) is convenient and inexpensive, and can often detect common problems associated with hemodialysis access. Routine systematic physical examination of the fistula by the dialysis staff with each treatment may allow early detection of problems that are commonly associated with mature fistula. This avoiding missed treatments and emergent situations. Dialysis access stenosis is the most common cause of access dysfunction. Physical examination is an important method in the assessment of stenotic lesions. The purpose of this study is to evaluate the two simple maneuvers in physical examination of the AVF (pulse augmentation and pressure assessment inside the fistula and collapsibility of the fistula on arm elevation) and compare them with the gold standard angiography.

Design, setting, participants, & measurements: This is a prospective cohort study of 118 consecutive hemodialysis patients who were referred to dialysis access center of Pittsburgh, PA because of dysfunctional AVF. We compared the accuracy of the clinical examination in diagnosing outflow stenosis in AVF with the gold standard (angiography). Two separate experienced interventional nephrologists (IN) were involved in the study. The IN who carried out the angiography of the fistula was blinded to the results of the physical examination findings. Cohen's k was used as a measurement of the level of agreement beyond chance between the physical examination and the angiography.

Results: There was good agreement between physical examination and angiography in the diagnosis of outflow stenosis (k value = 0.74). The sensitivity, specificity, positive and negative predicted values of the 2 maneuvers used in physical examination (augmentation, collapsibility of the fistula) were 94.3%, 79.1%, 86.4%, 90.3% and 93.3%, 79.5%, 88.5%, and 87.5%, respectively.

Conclusion: This study confirmed that physical examination of hemodialysis AVF can accurately diagnose outflow stenoses in mature fistula and correlated well with angiographic findings.

Keywords: AVF (arteriovenous fistula), angiography, augmentation of the AVF, collapsibility of the AVF, interventional nephrologist, stenosis of the outflow tract of the fistula.

Author α σ: Dialysis Access Center of Pittsburgh, PA.
e-mails: elmagbri@hotmail.com, elmagbri@gwmail.gwu.edu
Author ρ: Candidate for MD, George Washington School of Medicine, Washington, DC.
Author ω: BS candidate, American University, Washington, DC.
Author ¥: BS candidate, University of Pennsylvania.
e-mail: tahaelmagbri@yahoo.com

I. Background and Objectives

Physical examination of the hemodialysis arterial-venous fistula (AVF) is convenient and inexpensive, and can often detect common problems associated with hemodialysis access (1-5).

Routine physical examination of the fistula by the dialysis staff with each treatment may allow early detection of problems that are commonly associated with mature fistula, thus avoiding missed treatments and emergent situations. Dialysis access stenosis is the most common cause of access dysfunction. Therefore, physical examination is an important method in the assessment of stenotic lesion (1,6-9).

The 2006 National Kidney Foundation Kidney Disease Outcomes Quality Initiative (NKF-K/ DOQI) guidelines recommend that physical examination (monitoring) be performed on all mature AVFs on a weekly basis (10,11). Such monitoring is also recommended by the 2008 Society for Vascular Surgery practice guidelines (12). We strongly agree that hemodialysis AVF should be examined at every hemodialysis treatment. This requires that all clinical staff who are directly involved in the care of hemodialysis patients be familiar with the basic techniques used to examine the fistula.

The purpose of this study is to evaluate the two simple maneuvers in physical examination of the AVF (pulse augmentation and pressure assessment inside the fistula and collapsibility of the fistula on arm elevation) compared to the gold standard (angiography).

II. Subjects and Methods

A total of 118 patients dialyzed via a mature AVF were included. The patients were referred to the dialysis access center of Pittsburgh because of dysfunctional AVF. There were 27 right arm fistulas (3 radial-cephalic and 24 upper arms AVF), and 91 left arms AVF (15 radial-cephalic AVF, and 76 upper arms AVF), Table-1. The age range of the patients is 22 yrs to 92 yrs, with a mean of 63.2 yrs. 55% of the patients were males and 53% were diabetics. 91% of patients were hypertensive, and 4.3% have peripheral arterial disease.

Clinical examination of the dialysis AVF includes;

1. Pulse augmentation and pressure assessment in the fistula is graded into 1,2
2. Good augmentation of the pulse pressure and AVF is soft by palpation.
3. No augmentation and high pulse pressure in the AVF
4. Collapsibility of the fistula on arm elevation is also graded to;
5. The AVF is completely collapsed on arm elevation
6. The AVF is hyperpulsatile and not collapsed on arm elevation.

Pulse augmentation is assessed by complete occlusion of the access several centimeters away from the arterial anastomosis and evaluation of the stenght of the pulse as well as palpating the fistula without obstructing the outflow tract and assesses the pressure inside the fistula. The fistula is considered normal when there is good augmentation of the pulse upstream from the occluded finger (7). The pulse pressure as assessed by palpation is not increased in this case.

Collapsibility of the AVF is assessed by elevating the arm of the fistula above the heart and examination of the normal collapsing of the fistula. These two simple maneuvers are correlated with the angiogram findings of the AVF (7). The test was considered abnormal when the fistula remained pump after arm elevation. Then angiography is used to assess the fistula. Both retrograde and antegrade angiography were done to evaluate the access from the feeding artery to the right atrium (C-arm 9900 vascular package; General Electric, Milwaukee WI).

Two interventional nephrologists (IN) were involved, separately, in physical examination and angiographic examination and interpretation. To offset the bias, the IN who is carrying out the angiographic studies does not know about the results of the physical examination. The findings of the physical examination and angiography were then analyzed at the end of the study.

a) Statistical Analyses

Chi-square with Fisher's exact test for the two-tailed p value was used to analysis the dichotomous data from the physical examination and angiographic findings. A p value of $<.05$ was considered as significant. The Cohen's k value was used to measure the level of agreement beyond chance between the diagnoses made by physical examination and angiography (13,14,15). It is a robust statistic tool useful for either interrater or intrarater reliability testing. It can range from -1 to +1, where 0 represents the amount of agreement that can be expected from random chance, and 1 represents perfect agreement between the raters. Kappa value of <0 as indicating no agreement and 0.01-0.20 as none to slight, 0.21-0.40 as fair, 0.41-0.60 as moderate, 0.61-0.80 as substantial, and 0.81-1.00 as most perfect agreement. The kappa was designed to take account of the possibility of guessing.

III. Results

In this study only the outflow stenosis was assessed and compared to the finding on angiography. 74 patients were found to have significant out flow stenoses by angiography (>50% stenosis). Physical examination using collapsibility of the AVF detected 69/74 stenoses (93.3%), and augmentation and pulse pressure assessment detected 66/70 patients (94.3%), (Tables -2, 3). The specificity of the augmentation and collapsibility were 79.1% and 79.5%, respectively. Collapsing of the fistula missed 5 patients who had side branches to divert blood away from the main fistula.

Analysis of forearm and upper arm fistulas revealed no significant difference in the diagnostic accuracy of these two physical examination maneuvers in detecting stenosis. Sensitivity and specificity of forearm and upper arm fistula were identical. Therefore, no breakdown of the results by fistula type was done.

The sensitivity, specificity, positive and negative predictive value of the 2 maneuvers was calculated and is shown in Tables-2, 3.

The overall sensitivity of the augmentation and pulse pressure palpation when compared to angiography is 94.3%, with specificity of 79.1%, positive predictive value of the test of 86.4%, and negative predictive value of 90.3%, Table-2. The p value of the two-tailed fisher's test was highly significant <0.0001. There was a good agreement beyond chance between the physical examination and the angiography in the diagnosis of outflow stenosis (Cohen's k value for agreement $k=0.749$).

When collapsibility of the fistula is compared with angiography, the overall sensitivity of the maneuver is 93.3%, with specificity of 79.5%, positive and negative predictive values of 88.5% and 87.5% respectively, Table-3. The p value of the two-tailed fisher's test was highly significant <0.0001. There was a good agreement beyond chance between the physical examination and the angiography in the diagnosis of outflow stenosis (Cohen's k value for agreement $k=0.742$).

IV. Discussion

Physical examination is a good and convenient tool in the assessment of vascular access dysfunction. A few reports have studied and evaluated its usefulness in the detection of access stenosis when compared to the gold standard, angiography, (9,15). The results of this study agreed with the work of Choi et al (8), and Mishler et al (16). These investigators found that physical examination reliably diagnosed significant outflow stenosis of the AVF when compared to angiography. While, these workers showed the strength

of physical examination, their work was limited by co-founders; like study design, the sample size, lack of independent assessment of the angiographic images, and bias, since the same physician who performed the physical examination read the angiography images. Also, they did not report on the sensitivity, specificity of the physical examination, nor the agreement between the physical examination and angiography.

Both this study and that of Asif et al, avoided all these cofounders (8). Our study and that of Asif have clearly shown that physical examination has high sensitivity, specificity, and can be a useful tool for detecting stenosis in the dialysis access. We used Cohen's k values to ascertain the agreement between the physical examination and angiography. We found a robust correlation between physical examination and angiographic findings.

We undoubtedly, demonstrated high sensitivity and specificity (93 to 94% and 79%, respectively) of the physical examination to detect significant outflow stenosis in the dialysis access (AVF). The high sensitivity and specificity make physical examination a valuable tool to screen for the presence of out flow stenosis in mature AVF. This makes physical examination a valuable tool for streamline patients with dysfunctional fistula to vascular access center by the staff in a timely manner. Because physical examination of the vascular access is inexpensive and available, it should be adopted, universally, by all staff members who care for hemodialysis patient.

Performing physical examination during angioplasty of the stenosis can assist in the success of balloon angioplasty. It can also help the interventionalist as to the site of cannulation, thus, potentially save time, minimize morbidity, and reduce cost.

The limitations of this study are that physical examinations are carried out by well versed interventionalist who has long experience on vascular access evaluation. This may not be applied for the general nephrologists who often see the patients on the dialysis machine. The study also investigated only outflow obstruction in mature fistula as related to physical examination. Since the study has a small sample size, and was carried out in one facility may limit its applicability to all other dialysis facilities.

V. Conclusion

Dialysis access stenosis is the most common cause of access dysfunction. Physical examination of the hemodialysis vascular access is inexpensive and valuable tool in the diagnosis and localization of stenosis. Referring patients with dysfunctional access can avoid missed treatments, emergent situations, and can impact cost and inconvenience.

Table 1 : Location of arterial-venous fistulas

	RCAVF	Upper arm AVF
Right arm	3	24
Left arm	15	76

RCAVF- radial-cephalic arterial-venous fistula

Table 2 : Augmentation of fistula and pulse pressure Vs Angiography

	Angiography positive	Angiography negative	
No Augmentation & high pressure	66	10	PPV = 86.4%
Good augmentation & fistula soft	4	38	NPV = 90.3%
	Sensitivity = 94.3%	Specificity = 79.1%	Prevalence = 59.3%

PPV = Positive predictive value
NPV = Negative predicative value
Two-tailed Fisher's exact test (p value <0.0001)
(Cohen's k value for agreement k=0.749).

Table 3 : Collapsibility of AVF Vs Angiography

	Angiography positive	Angiography negative	
AVF not collapsible	69	9	PPV = 88.5%
AVF collapsible	5	35	NPV = 87.5%
	Sensitivity = 93.3%	Specificity = 79.5%	Prevalence = 62.7%

PPV = Positive predictive value
NPV = Negative predictive value
Two-tailed Fisher's exact test (p value <0.0001)
(Cohen's k value for agreement k=0.742).

References Références Referencias

1. Beathard GA. Physical examination of the dialysis vascular access. Semin Dial 1998; 11:231
2. Trerotola SO, Scheel PJ Jr, Powe NR, et al Screening for dialysis access graft malfunction: comparison of physical examination with US. J Vasc Interv Radiol 1996; 7:15
3. Safa AA, Valji K, Roberts AC, et al. Detection and treatment of dysfunctional hemodialysis access graft: effect of a surveillance program on graft

patency and the incidence of thrombosis. Radiology 1996; 199: 653.
4. Migliacci R, Selli ML, Falcinelli F, et al. Assessment of occlusion on the vascular access in patients on chronic hemodialysis: comparison of physical examination with continuous-wave Doppler Ultrasound. STOP Investigators. Shunt Thrombotic Occlusion Prevention with Picotamide. Nephron 1999; 82: 7.
5. Asif A, Leon C, Orozco-Vargas LC, et al. Accuracy of physical examination in the detection of Arteriovenous fistula stenosis. Clin J Am Soc Nephrol 2007; 2: 1191.
6. Beathard GA. Physical examination: The forgotten tool. In: A Multidisciplinary Approach for Hemodialysis Access, edited by Gray R, Sands J, New York, Lippincott Williams & Wilkins, 2002, pp 11-118.
7. Beathard GA: An algorithm for the physical examination of early fistula failure. Semin Dial 18: 331-335, 2005.
8. Choi JR, Kim YS, Yoon SA, Won YD, et al. Accuracy of physical examination in the detection of Arteriovenous fistula dysfunction. Korean J Nephrol 25; 797-802, 2006.
9. Asif A, Cherla G, Merrill D, Cipleu CD, Briones P, Pennell P. Conversion of tunneled hemodialysis catheter-consigned patients to Arteriovenous fistula. Kidney Int 67: 2399-2406. 2005.
10. Vascular Access Work Group. Clinical practice guidelines for vascular access. Am J Kidney Dis 2006; 48 (suppl 1): S248.
11. NKF-DOQI clinical practice guidelines for vascular access: guideline 1-Patient evaluation prior to access placement. Am J Kidney Dis 37 (suppl): S141; 2001.
12. Sidawy AN, Spergel LM, Besarab A, et al. The Scociety for Vascular Surgery: clinical practice guidelines for surgical placement and maintenance of Arteriovenous hemodialysis access. J Vasc Surg 2008; 48: 2S.
13. McGinn T, Wyer PC, Newman TB, Keitz S, et al. Tips for learners of evidence-based medicine: Measures of observer variability (kappa statistic). CMAJ 171: 1369-1373, 2004.
14. Maclure M, Willett WC: Misinterpretation and misuse of the kappa statistics. Am J Epidemiology 126: 161-169, 1987.
15. Cohen J. A coefficient of agreement for nominal scales. Educ Psychol Meas. 1960; 20: 37-46.
16. Mishler R, Schon D, Hubert B, Nissenson AR. Development and usefulness of a physical examination tool to diagnose vascular access dysfunction. J AM Soc Nephrol 2000; 11: 190A.

Global Journal of Medical Research: F
Diseases
Volume 16 Issue 3 Version 1.0 Year 2016
Type: Double Blind Peer Reviewed International Research Journal
Publisher: Global Journals Inc. (USA)
Online ISSN: 2249-4618 & Print ISSN: 0975-5888

Knowledge, Attitude and Practices towards Noncommunicable Disease Risk Factors among Medical Staff

By Dr. Suadad j. AL-Daboony
Al-Basrah University

Abstract- *Background:* The Iraq suffers from a high burden of noncommunicable diseases (NCD). Iraqi people have a high prevalence of child obesity, adolescent and adult obesity, diabetes, heart disease and cancers among its adult population.

Objective: To identify knowledge, attitude and practices relating to modifiable noncommunicable disease (NCD) risk factors regarding medical staff that includes doctors, dentists and pharmacists.

Methods: the study conducted from January 2015 to December 2015, the study was carried out by using questionnaires. Data were analyzed using SPSS statistical software version 20.

Overall 70.2% of the participants reported no practiced physical exercise.

High proportions of both males and females no practiced of physical activity, especially physical activity in their leisure time. However, the percentages of daily vigorous, moderate physical activity were low for both males and females. Low proportions of males and females reported daily intakes of fruits and vegetables.

Keywords: *knowledge, HIV/AIDS, floating population and bangladesh.*

GJMR-F Classification : *NLMC Code: WD300*

Strictly as per the compliance and regulations of:

Knowledge, Attitude and Practices towards Noncommunicable Disease Risk Factors among Medical Staff

Dr. Suadad j. AL-Daboony

Abstract- Background: The Iraq suffers from a high burden of noncommunicable diseases (NCD). Iraqi people have a high prevalence of child obesity, adolescent and adult obesity, diabetes, heart disease and cancers among its adult population.

Objective: To identify knowledge, attitude and practices relating to modifiable noncommunicable disease (NCD) risk factors regarding medical staff that includes doctors, dentists and pharmacists.

Methods: the study conducted from January 2015 to December 2015, the study was carried out by using questionnaires. Data were analyzed using SPSS statistical software version 20.

Results: We obtained data from 524 participants, 200 male (38.2%) & 324 female (61.8%). The age distribution of participant under 30 years 161(30.7%), 31 to 40 years 154(29.4), 41 to 50 years 121(23.1%) and over 50 years 88 (16.8%). From the total sample of 524, 106 dentists (20.2%), 81 pharmacists (15.5%), 185 general practitioners (35.3%) and 152 specialist (29%).

Overall 70.2% of the participants reported no practiced physical exercise.

High proportions of both males and females no practiced of physical activity, especially physical activity in their leisure time. However, the percentages of daily vigorous, moderate physical activity were low for both males and females. Low proportions of males and females reported daily intakes of fruits and vegetables.

According to body mass index percentage of overweight and obesity of the participants 42.9%, 21.6 respectively.

A majority of participants had knowledge about risk factors of non-communicable disease but there were shortage in practice.

Discussion: This study contributes knowledge, attitude, and practice towards NCD risk factors with a focus on practical point. No significant gender differences were found in physical activity practices. Results that a majority of participants was physically inactive and have poor nutritional intakes.

Keywards: knowledge- attitude & practice (KAP), noncommunicable disease (NCD), Risk factors (RF).

I. Introduction

Knowledge is a set of understandings, having information, comes from experience or education (having knowledge means having extensive information or understanding).

Author: FICMS-Community Medicine, M.B.Ch.B - College of Medicine/ Al-Basrah University. e-mail: suadad2025@gmail.com

Attitude is a way of being, a position. These are learning this is an Intermediate variable between the situation, and the response to this situation.

Practices this is something that deals with the concrete, with actions, practices or behaviors are the observable actions of an individual in response to a stimulus. This is something that deals with the concrete, with actions.

A KAP (knowledge, Attitude and Practice) survey is conducted to investigate human behavior related to a certain topic. It identifies what people know (Knowledge), how they feel (Attitude) and what they do (Practice).

II. Noncommunicable Disease

Chronic non communicable disease; in USA has defined chronic disease as comprising all impairments or deviations from normal, which have one or more of the fallowing characteristics:

- Impairment
- Leave residual disability
- Are caused by non reversible pathological alteration
- Required social training of the patient rehabilitation
- May be expected to require long period of supervision, observation or care

Non communicable diseases (NCDs) includes cardiovascular disease, renal, nervous and mental disease, musculoskeletal condition, respiratory disease, permanent result of accidents. Chronic non-communicable disease are assuming increasing importance among the adult population in both developed and developing countries [(1)]

Noncommunicable diseases (NCDs), also known as chronic diseases, are not passed from person to person. They are of long duration and generally slow progression. The four main types of noncommunicable diseases are cardiovascular diseases (like heart attacks and stroke), cancers, chronic respiratory diseases (such as chronic obstructed pulmonary disease and asthma) and diabetes [(2)].

Today, noncommunicable diseases (NCDs), are responsible for more than 75% of deaths worldwide [(3)]

The economic consequences of noncommunicable diseases are huge, because of the combined burden of health care costs and lost

economic productivity due to illness and premature death.[4]

In most countries, people who have a low socioeconomic status and those who live in poor or marginalized communities have a higher risk of dying from non-communicable diseases (NCDs) than do more advantaged groups and communities. Smoking rates, blood pressure, and several other NCD risk factors are often higher in groups with low socioeconomic status than in those with high socioeconomic status; the social gradient also depends on the country's stage of economic development, cultural factors, and social and health policies. [5]

Noncommunicable disease "lifestyle" diseases because the majority of risk factors were preventable, illnesses from smoking, alcohol abuse, poor diets and physical inactivity killed some 36 million people a year, mostly in low and middle-income countries where they disproportionately affected people under 60.[6]

a) Non-Communicable Disease Risk Factors

Risk factors such as a person's background; lifestyle and environment are known to increase the likelihood of certain non-communicable diseases. They include age, gender, genetics, exposure to air pollution, and behaviors such as smoking, unhealthy diet and physical inactivity which can lead to hypertension and obesity, in turn leading to increased risk of many NCDs. Most NCDs are considered preventable because they are caused by modifiable risk factors.

Most epidemiologists accept that sex key set of risk factors are of adult non-communicable disease morbidity and mortality these as fallow

- Cigarette use and other form of smoking.
- Alcohol abuse
- Failure or inability to obtain preventive health services (e.g. for hypertension control, cancer detection, management of diabetes)
- Life style changes (e.g. dietary patterns, physical activity)
- Environmental risk factors (e.g. occupational hazards. Air and water pollution possession of destructive weapons in case of injury,
- Stress factors. [1]

Common, preventable risk factors underlie most noncommunicable diseases. Most noncommunicable diseases are the result of four particular behaviors (tobacco use, physical inactivity, unhealthy diet, and the harmful use of alcohol) that lead to four key metabolic/ physiological changes (raised blood pressure, overweight/obesity, raised blood glucose and raised cholesterol.[7]

The hazardous effects of behavioral and dietary risk factors on noncommunicable diseases, and the metabolic and physiological conditions that mediate their effects.

There is less information on risk-factor trends, which makes it difficult to assess how they have affected population health in the past or how they may do so in the future.[8]

Noncommunicable diseases (NCDs) are a major disease burden in the Region. Many of the risk factors are related to lifestyle and can be controlled. Physical inactivity, low fruit and vegetable intake, high fast food consumption and high cholesterol are predominant causes of cardiovascular disease and some cancers. Overweight and obesity can lead to metabolic changes and raise the risk of NCDs, including heart disease and type 2 diabetes.(9)

b) Tobacco

The hazardous effects of smoking on mortality from cancers and cardiovascular and respiratory diseases have been known for decades [8]

Tobacco products are products made entirely or partly of leaf tobacco as raw material, which are intended to be smoked, sucked, chewed or snuffed. All contain the highly addictive psychoactive ingredient, nicotine.

Tobacco use is one of the main risk factors for a number of chronic diseases, including cancer, lung diseases, and cardiovascular diseases. [10]

The tobacco epidemic is one of the biggest public health threats the world has ever faced, killing around 6 million people a year. More than 5 million of those deaths are the result of direct tobacco use while more than 600 000 are the result of non-smokers being exposed to second-hand smoke. [2]

The majority of the more than 1 billion smokers worldwide now live in low- and middle-income countries. [8]

On the basis of current smoking patterns, with a global average of about 50% of young men and 10% of young women becoming smokers and relatively few stopping, annual tobacco-attributable deaths will rise from about 5 million in 2010 to more than 10 million a few decades.

Tobacco is the biggest external cause of noncommunicable disease and is responsible for even more deaths than adiposity both in high-income countries such as the United States and globally. The risks in middle age are much greater for smokers who started in early adulthood than for those who started later. This means that the ratio of mortality among smokers to that among persons who have never smoked is much more extreme now. [11]

c) Physical Activity

WHO defines physical activity as any bodily movement produced by skeletal muscles that require energy expenditure – including activities undertaken while working, playing, carrying out household chores, travelling, and engaging in recreational pursuits?

The term "physical activity" should not be confused with "exercise", which is a subcategory of physical activity that is planned, structured, repetitive, and aims to improve or maintain one or more components of physical fitness. Both, moderate and vigorous intensity physical activity brings health benefit.[12]

Studies of the beneficial health effects of physical activity date back to the 1950s and have been replicated in large cohorts. Physical activity at work, walking, and, in some populations, bicycling used to be major contributors to total energy expenditure but have declined dramatically in industrial and urban societies. Paralleling this shift, more recent epidemiologic studies in high-income countries have focused on leisure-time activity, with less emphasis on work and methods of local transportation, which are important in developing countries. Only recently has attention been given to population-based measurement of physical activity in countries at all stages of urbanization and economic development. The limited available global data nonetheless show low levels of activity and long periods in sedentary conditions in high-income and urbanized countries and higher activity levels in rural populations that engage in agricultural activity and walk or bicycle long distances for daily activities.[8]

Physical activity recommendations for specific age groups

The "Global Recommendations on Physical Activity for Health" address three age groups: 5–17 years old, 18–64 years old and 65 years old and above. These age groups were selected taking into consideration the nature and availability of the scientific evidence relevant to the prevention of noncommunicable diseases through physical activity.

Physical activity recommended amount about Children and adolescents aged 5-17 years

- Should do at least 60 minutes of moderate to vigorous-intensity physical activity daily.
- Physical activity of amounts greater than 60 minutes daily will provide additional health benefits.
- Should include activities that strengthen muscle and bone.

Adults aged 18–64 years

- Should do at least 150 minutes of moderate-intensity physical activity throughout the week, or do at least 75 minutes of vigorous-intensity physical activity throughout the week, or an equivalent combination of moderate- and vigorous-intensity activity.[12]

Regular physical activity is one of the most important things you can do for your health. It can help: Control your weight, Reduce your risk of cardiovascular disease, Reduce your risk for type 2 diabetes and metabolic syndrome, Reduce your risk of some cancers, Strengthen your bones and muscles, Improve your mental health and mood, Improve your ability to do daily activities and prevent falls, if you're an older adult, Increase your chances of living longer. [13]

Physical inactivity is an important behavioral risk factor that is associated with many negative health consequences. The health benefit of regular physical activity relates to an improved quality of life and reduces the risk of a variety of disorders. [14]

Physical inactivity is a modifiable risk factor for cardiovascular disease and a widening variety of other chronic diseases, including diabetes mellitus, cancer (colon and breast), obesity, hypertension, bone and joint diseases. [15]

Meeting the 2008 Physical Activity Guidelines for Americans minimum by either moderate- or vigorous-intensity activities was associated with nearly the maximum longevity benefit. We observed a benefit threshold at approximately 3 to 5 times the recommended leisure time physical activity minimum and no excess risk at 10 or more times the minimum. [16]

Higher cardio- respiratory fitness (CRF) and physical activity (PA) in old age are associated with greater brain structural and functional integrity, and higher cognitive functioning. [17]

Aerobic activity or "cardio" gets you breathing harder and your heart beating faster. From pushing a lawn mower, to taking a dance class, to biking to the store – all types of activities count. As long as you're doing them at a moderate or vigorous intensity for at least 10 minutes at a time.

Intensity is how hard your body is working during aerobic activity.

How do you know if you're doing light, moderate, or vigorous intensity aerobic activities?

For most people, light daily activities such as shopping, cooking, or doing the laundry doesn't count toward the guidelines. Why? Your body isn't working hard enough to get your heart rate up.

Moderate-intensity aerobic activity means you're working hard enough to raise your heart rate and break a sweat. One way to tell is that you'll be able to talk, but not sing the words to your favorite song. Here are some examples of activities that require moderate effort: Walking fast, Doing water aerobics, Riding a bike on level ground or with few hills, Playing doubles tennis, Pushing a lawn mower.

Vigorous-intensity aerobic activity means you're breathing hard and fast, and your heart rate has gone up quite a bit. If you're working at this level, you won't be able to say more than a few words without pausing for a breath. Here are some examples of activities that require vigorous effort: Jogging or running, Swimming laps, Riding a bike fast or on hills, Playing singles tennis, Playing basketball. [18]

d) Unhealthy Diet

An unhealthy diet is one of the major risk factors for a range of chronic diseases, including cardiovascular diseases, cancer, diabetes and other conditions linked to obesity. Specific recommendations for a healthy diet include eating more fruit, vegetables, legumes, nuts and grains; cutting down on salt, sugar and fats. It is also advisable to choose unsaturated fats, instead of saturated fats and towards the elimination of trans-fatty acids.

Improving dietary habits is a societal, not just an individual problem. Therefore, it demands a population-based, multispectral, multi-disciplinary, and culturally relevant approach. [19]

A healthy diet helps protect against malnutrition in all its forms, as well as noncommunicable diseases (NCDs), including diabetes, heart disease, stroke and cancer. Unhealthy diet and lack of physical activity are leading global risks to health.

Healthy dietary practices start early in life – breastfeeding fosters healthy growth and improves cognitive development, and may have longer-term health benefits, like reducing the risk of becoming overweight or obese and developing NCDs later in life. Energy intake (calories) should be in balance with energy expenditure. Evidence indicates that total fat should not exceed 30% of total energy intake to avoid unhealthy weight gain, with a shift in fat consumption away from saturated fats to unsaturated fats, and towards the elimination of industrial trans fats. Limiting intake of free sugars to less than 10% of total energy intake is part of a healthy diet.

A further reduction to less than 5% of total energy intake is suggested for additional health benefits .Keeping salt intake to less than 5 g per day helps prevent hypertension and reduces the risk of heart disease and stroke in the adult population. WHO Member States have agreed to reduce the global population's intake of salt by 30% and halt the rise in diabetes and obesity in adults and adolescents as well as in childhood overweight by 2025. [20]

Consuming a healthy diet throughout the life course helps prevent malnutrition in all its forms as well as a range of noncommunicable diseases and conditions. However, the increased production of processed food, rapid urbanization and changing lifestyles have led to a shift in dietary patterns.

People are now consuming more foods high in energy, fats, free sugars or salt/sodium, and many do not eat enough fruit, vegetables and dietary fiber such as whole grains.

The exact make-up of a diversified, balanced and healthy diet will vary depending on individual needs (e.g. age, gender, lifestyle, degree of physical activity), cultural context, locally available foods and dietary customs. [20]

It appears conceivable that the risk of hypercholesterolemia can be reduced by changing the snack dietary pattern. [21]

e) Alcohol

Alcohol consumption is associated with numerous diseases and injuries. Moderate alcohol consumption has been inversely associated with the risk of cardiovascular diseases and diabetes, although the benefits may be greater for persons with existing cardiovascular risk factors than for those without such risk factors. Epidemiologic studies that have measured both the amount and patterns of alcohol consumption have shown that heavy episodic (or binge) drinking not only substantially raises the risk of injuries but can also increase the risk of or exacerbate cardiovascular disease and liver disease. [8]

In many parts of the world, drinking alcoholic beverages is a common feature of social gatherings. Nevertheless, the consumption of alcohol carries a risk of adverse health and social consequences related to its intoxicating, toxic and dependence-producing properties. [22]

Alcohol is a psychoactive substance with dependence-producing properties that has been widely used in many cultures for centuries. The harmful use of alcohol causes a large disease, social and economic burden in societies. Environmental factors such as economic development, culture, availability of alcohol and the level and effectiveness of alcohol policies are relevant factors in explaining differences and historical trends in alcohol consumption and related harm.

The volume of alcohol consumed, the pattern of drinking determines alcohol-related harm, and, on rare occasions, the quality of alcohol consumed. The harmful use of alcohol is a component cause of more than 200 disease and injury conditions in individuals, most notably alcohol dependence, liver cirrhosis, cancers and injuries. [23]

Excessive alcohol use can lead to the development of chronic diseases and other serious problems including high blood pressure, heart disease, stroke, liver disease, and digestive problems. Cancer of the breast, mouth, throat, esophagus, liver, and colon., Learning and memory problems, including dementia and poor school performance., Mental health problems, including depression and anxiety., Social problems, including lost productivity, family problems, and unemployment. Alcohol dependence, or alcoholism. [24]

f) Obesity

Overweight and obesity are defined as abnormal or excessive fat accumulation that presents a risk to health. A crude population measure of obesity is the body mass index (BMI), a person's weight (in kilograms) divided by the square of his or her height (in meters). A person with a BMI of 30 or more is generally

considered obese. A person with a BMI equal to or more than 25 is considered overweight.

Overweight and obesity are major risk factors for a number of chronic diseases, including diabetes, cardiovascular diseases and cancer. Once considered a problem only in high income countries, overweight and obesity are now dramatically on the rise in low- and middle-income countries, particularly in urban settings. (25)

Owing to the increasing obesity trends, our findings suggest that in 20 years an increasing number of people will be living with an obesity-related chronic disease in almost every country in Europe. (26)

g) Prevention

i. *Prevention and control of noncommunicable diseases (NCDs)*

Non modifiable risk factors of noncommunicable disease: age, sex, races &genetic factors.

Modifiable risk factors: smoking, alcohol, unhealthy diet, physical inactive, stress. (1)

To lessen the impact of NCDs on individuals and society, a comprehensive approach is needed that requires all sectors, including health, finance, foreign affairs, education, agriculture, planning and others, to work together to reduce the risks associated with NCDs, as well as promote the interventions to prevent and control them.

An important way to reduce NCDs is to focus on lessening the risk factors associated with these diseases. Low-cost solutions exist to reduce the common modifiable risk factors (mainly tobacco use, unhealthy diet and physical inactivity, and the harmful use of alcohol) and map the epidemic of NCDs and their risk factors.

Other ways to reduce NCDs are high impact essential NCD interventions that can be delivered through a primary health-care approach to strengthen early detection and investments because, if applied to patients early, can reduce the need for more expensive treatment. These measures can be implemented in various resource levels. The greatest impact can be achieved by creating healthy public policies that promote NCD prevention and control and reorienting health systems to address the needs of people with such diseases.

Lower-income countries generally have lower capacity for the prevention and control of noncommunicable diseases.

High-income countries are nearly four times more likely to have NCD services covered by health insurance than low-income countries. Countries with inadequate health insurance coverage are unlikely to provide universal access to essential NCD interventions. (2)

The behaviors of individuals are important factors in the patterns of risk factors for noncommunicable diseases, successful efforts to reduce smoking, alcohol consumption, and, more recently, trans-fat and salt consumption show that there is great scope for collective action through policy formulation and implementation successful policies, such as tobacco and alcohol taxes and restrictions, should be replicated in all populations. There is also a need for bold and creative policies that address harmful alcohol consumption, improve diet, and increase physical activity. (8)

With respect to reducing mortality, advances in cancer treatment have not been as effective as those for other chronic diseases; effective screening methods are available for only a few cancers. Primary prevention through lifestyle and environmental interventions remains the main way to reduce the burden of cancers.

Smoking, alcohol use, and low fruit and vegetable intake were the leading risk factors for death from cancer worldwide and in low-and-middle-income countries. In high-income countries, smoking, alcohol use, and overweight and obesity were the most important causes of cancer. (27)

The products of tobacco, alcohol and food industries are responsible for a significant and growing proportion of the global burden of disease. Smoking and alcohol combined account for 12.5% of global deaths and 19.5% in high-income countries, while six diet-related risk factors account for 13.6 and 17.5% of deaths, respectively.

Arguably, the greatest challenge and opportunity for public health lies in reducing the contributions of tobacco use, unhealthy diet and harmful alcohol consumption to the rising global burden of non-communicable diseases. This demonstrates a pressing need to improve our understanding of how corporations contribute to this disease burden, both directly through the promotion of products damaging to health and indirectly through influence over public policy. The concept of an industrial epidemic—an epidemic emerging from the commercialization of potentially health-damaging products—lends itself to this purpose. Adapting traditional public health constructs, it identifies the role of the host (the consumer), agent (the product, e.g. cigarettes, alcohol), environment and, crucially, the disease vector (the corporation). (28)

Elevation of blood cholesterol concentrations has been recognized as a major risk factor for cardiovascular diseases. Control of the increase in blood cholesterol is one of the important strategies for the prevention of cardiovascular diseases. (29)

A healthy diet contains:

Fruits, vegetables, legumes (e.g. lentils, beans), nuts and whole grains (e.g. unprocessed maize, millet,

oats, wheat, brown rice).At least 400 g portions) of fruits and vegetables a day .

Potatoes, sweet potatoes, cassava and other starchy roots are not classified as fruits or vegetables. Less than 10% of total energy intake from free sugars (which is equivalent to 50 g (or around 12 level teaspoons) for a person of healthy body weight consuming approximately 2000 calories per day, but ideally less than 5% of total energy intake for additional health benefits . Most free sugars are added to foods or drinks by the manufacturer, cook or consumer, and can also be found in sugars naturally present in honey, syrups, fruit juices and fruit juice concentrates. Less than 30% of total energy intake from fats , Unsaturated fats (e.g. found in fish, avocado, nuts, sunflower, canola and olive oils) are preferable to saturated fats (e.g. found in fatty meat, butter, palm and coconut oil, cream, cheese, ghee and lard) . Industrial trans fats (found in processed food, fast food, snack food, fried food, frozen pizza, pies, cookies, margarines and spreads) are not part of a healthy diet. Less than 5 g of salt (equivalent to approximately 1 teaspoon) per day and use iodized salt. [20]

III. Methods

Study conducted from January 2015 to December 2015 by using questionnaire, 524 participants (includes doctors, dentists and pharmacists) were randomly selected. The questions were created with consideration to some of the main NCD risk factors, physical inactivity, obesity and poor diet, smoking, alcohol consumption& because these risk factors are common to diabetes mellitus, some cancers and cardiovascular diseases (CVDs), all of which constitute the NCD health burden in Iraq. Traditions and cultural practices were also considered when formulating the questions.

A cross-sectional survey was created with both quantitative and qualitative questions.

A majority of the questions focused on physical activity: the type of physical activity practiced in the work environment and in leisure time; socio-cultural factors that influenced, also question about diet (vegetable and fruit intake), alcohol consumption, stress of life and questionnaire included personal data (age, gender, education, kind of work). The survey was tested with measured body mass index, overweight and obesity were often measured using the BMI (Body Mass Index) and according to body mass index scale.

BMI: is a simple index commonly used to classify overweight and obesity in schoolchildren and adults;
is calculated as a person's weight (in kg) divided by his or her height (in m2); Underweight: < 18.

Normal weight: 18.5 - 24.9

Overweight: 25 - 29.9

Obese: ≥ 30and does not distinguish weight associated with muscle from weight associated with fat and therefore provides only a crude measure of fatness

a) Data Analysis

The data was entered and analyzed using Statistical Package for the Social Science (SPSS) Version 20 statistical analysis program, Chi-square test was used to determine the significance of association between the variables

IV. Results

a) Demographic Characteristics

We obtained data from 524 participants, 200 male (38.2%) & 324 female (61.8%) are listed in the table (1)

Table (1) : Gender

	Frequency	Percent	Cumulative Percent
male	200	38.2	38.2
female	324	61.8	100.0
Total	524	100.0	

The age distribution of participant under 30 years 161(30.7%), 31 to 40 years 154(29.4%), 41 to 50 years 121(23.1%) and over 50 years 88(16.8%), the distribution of the age in the study are listed in the table(2).

Table (2) : Age

	Frequency	Percent	Cumulative Percent
Under 30 years	161	30.7	30.7
31-40 years	154	29.4	60.1
41-50 years	121	23.1	83.2
Over 50 years	88	16.8	100.0
Total	524	100.0	

From the total sample of 524, 106 dentists (20.2%), 81 placemats (15.5%), 185 general practitioners (35.3%) and 152 specialist (29%). the distribution of the jobs in the study are listed in the table (3).

Table (3) : Current Job

	Frequency	Percent	Cumulative Percent
dentists	106	20.2	20.2
pharmasts	81	15.5	35.7
general doctors	185	35.3	71.0
specialist	152	29.0	100.0
Total	524	100.0	

About the education of participants, 295(56.3%) bachelor s degree, 212(40.5%) post graduate and 17 (3.2%) other type of education, distribution of the education in the study are listed in the table(4).

Table (4) : Education

	Frequency	Percent	Cumulative Percent
Bachelor s degree	295	56.3	56.3
Specialized/Professional Graduate or post graduate	212	40.5	96.8
other	17	3.2	100.0
Total	524	100.0	

In the questionnaire there is question about NCD program have any idea about program or taken any workshop, lecture etc about non-communicable disease in the past 12 months.

About half of participant have idea about non communicable disease. only 122(23.3) was taken lecture, workshop about it, frequency distribution are listed in the table (5) &(6).

Table (5) : Have you heard about NCD program

	Frequency	Percent	Cumulative Percent
yes	264	50.4	50.4
no	260	49.6	100.0
Total	524	100.0	

Table (6) : In the past 12 months have lecture or workshop & so on

	Frequency	Percent	Cumulative Percent
YES	122	23.3	23.3
NO	402	76.7	100.0
Total	524	100.0	

behavioral risk factors of NCD; tobacco use, diet, physical activity, alcohol use, sedentary life.

Prevalence of participant's knowledge about noncommunicable disease risk factors 464(88.5) have knowledge about these, the distribution are listed in the table (7).

Table (7) : Risk Factors of NCD

Risk factors	Frequency	Percent	Cumulative Percent
smoking	18	3.4	3.4
sedentary life	3	.6	4.0
over weight	2	.4	4.4
no physical activity	10	1.9	6.3
unhealthy diet	3	.6	6.9
all of	464	88.5	95.4
non	24	4.6	100.0
Total	524	100.0	

Physical inactivity is a modifiable risk factor for cardiovascular disease and a widening variety of other chronic diseases, including diabetes mellitus, cancer (colon and breast), obesity, hypertension, bone and joint diseases (osteoporosis and osteoarthritis), and depression. The prevalence of physical inactivity among participant 368.2(70.2%).

Overall 70.2% of the participants reported no physical exercise, high proportions of both males and females no practiced of physical activity, especially physical activity in their leisure time. However, the percentages of daily vigorous physical activity, a component of total daily physical activity, were low for both males and females, distributions in the table (8), (9), (10), (11), (12),(13).

Table (8) : Have physical activity

	Frequency	Percent	Cumulative Percent
have physical activity	156	29.8	29.8
no physical activity	368	70.2	100.0
Total	524	100.0	

Table (9) : Vigorous Activity in the leisure time

	Frequency	Percent	Cumulative Percent
Yes	28	5.3	5.3
No	496	94.7	100.0
Total	524	100.0	

Table(10) : Days of Vigorous Activity in the leisure time

days	Frequency	Percent	Cumulative Percent
.00	496	94.7	94.7
1.00	8	1.5	96.2
2.00	15	2.9	99.0
3.00	2	.4	99.4
4.00	2	.4	99.8
5.00	1	.2	100.0
Total	524	100.0	

Table (11) : In the work have Vigorous Activity

	Frequency	Percent	Cumulative Percent
Yes	11	2.1	2.1
No	513	97.9	100.0
Total	524	100.0	

Table (12) : Moderate Activity in the leisure time

	Frequency	Percent	Cumulative Percent
Yes	154	29.4	29.4
No	370	70.6	100.0
Total	524	100.0	

Table (13) : Days of moderate avtivity

days	Frequency	Percent	Cumulative Percent
.00	370	70.6	70.6
1.00	31	5.9	76.5
2.00	51	9.7	86.3
3.00	28	5.3	91.6
4.00	14	2.7	94.3
5.00	19	3.6	97.9
7.00	11	2.1	100.0
Total	524	100.0	

Low proportion of females and males participated in physical activity in their leisure time. The low prevalence of leisure time physical activity is apparent throughout all age groups and no gender differences are found at the 0.05 level of significance (Table 14).). males reported higher percentages of daily physical activity33% of male compared 27% in female in table(14).

Although no significant age differences were found in terms of physical activity participation are listed in the table(15),

Table(14) : Crosstab

Count

		have physical activity		Total
		have physical activity	no physical activity	
Gender	male	(33%) 66	134	200
	female	(27%)90	234	324
Total		156	368	524

Gender * have physical activity

Chi-square=1.613 DF= 1 p-value 0.204

Table(15) : Crosstab between age & physical activity

		have physical activity		Total
		have physical activity	no physical activity	
Age	Under 30 years	51	110	161
	31-40 years	44	110	154
	41-50 years	36	85	121
	Over 50 years	25	63	88
Total		156	368	524

Chi-square 0.463 DF-3 p-value 0.937

The distribution of the risk factors (tobacco. alcohol intake in the study are listed in the table (16) & (17).

Table (16) : Tobacco Smoking

smoking	Frequency	Percent	Cumulative Percent
never smoke	435	83.0	83.0
previous smoke	31	5.9	88.9
current smoker, but not every day	32	6.1	95.0
currently a daily smoke	26	5.0	100.0
Total	524	100.0	

Table(17) : Consumption of alcohol

	Frequency	Percent	Cumulative Percent
Yes	16	3.1	3.1
No	508	96.9	100.0
Total	524	100.0	

Table(18) : How many fruits& vegetables

VEGETABLE-FRUITS	Frequency	Percent	Cumulative Percent
equal to 1 serving fruits - vegetables	255	48.7	48.7
more than one serving fruits - vegetables	159	30.3	79.0
only fruits	66	12.6	91.6
only vegetables	29	5.5	97.1
not eat fruits or vegetables	15	2.9	100.0
Total	524	100.0	

About awareness of checking blood pressure, blood sugar present in the table (19), high level of awareness by checking blood pressure and blood sugar.

Table (19) : checking blood pressure, blood sugar

	Frequency	Percent	Cumulative Percent
Yes	501	95.6	95.6
No	23	4.4	100.0
Total	524	100.0	

Prevention of noncommunicable disease, most of participants trying to lose weight ,doing physical activity and eating healthy diet 391(74.6%) have know important normal weight present in the table (20),(21)

Table (20)

prevention	Frequency	Percent	Cumulative Percent
lose weight, healthy diet, try to doing physical activity	391	74.6	74.6
no t trying	133	25.4	100.0
Total	524	100.0	

Table (21) : How important have normal weight

prevention		Frequency	Percent	Valid Percent	Cumulative Percent
Valid	not important	22	4.2	4.2	4.2
	important	185	35.3	35.3	39.5
	moderately important	68	13.0	13.0	52.5
	very important	249	47.5	47.5	100.0
	Total	524	100.0	100.0	

High percent trying to change weight 56.1% trying lose weight are listed in the table (22)

Table (22) : Are you trying to change weight

prevention	Frequency	Percent	Cumulative Percent
lose weight	294	56.1	56.1
gain weight	57	10.9	67.0
stay the same weight	147	28.1	95.0
not trying	26	5.0	100.0
Total	524	100.0	

In the study calculate body mass index for all participant found 182 (34.7%) normal weight, 225(42.9%) over weight and 113 (21.6%) obese are listed the table (23) & figure (1)

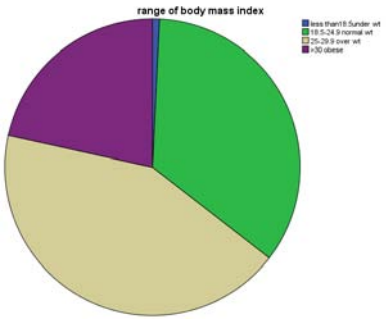
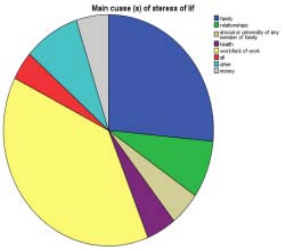


Figure (1)

Table (23) : Range of body mass index

BMI	Frequency	Percent	Cumulative Percent
less than18.5under wt	4	.8	.8
18.5-24.9 normal wt	182	34.7	35.5
25-29.9 over wt	225	42.9	78.4
>30 obese	113	21.6	100.0
Total	524	100.0	

Questionnaire include question asking participants about the most causes of stress on his or her life, the result are listed in the table (24) & figure (2).



In the figure (2) show more stressful factor of participants

Table (24) : Main (s) of stress of life

Stressful factors	Frequency	Percent	Cumulative Percent
family	139	26.5	26.5
relationships	42	8.0	34.5
school or university of any member of family	25	4.8	39.3
health	24	4.6	43.9
work/lack of work	202	38.5	82.4
all	21	4.0	86.5
other	45	8.6	95.0
money	26	5.0	100.0
Total	524	100.0	

Main reasons don't get physical activity (no time which the main reason equal to 73.1% are listed in the table (25)

Table (25) : Main reasons do not get exercise

Main cause don't get physical activity	Frequency	Percent	Cumulative Percent
no time	383	73.1	73.1
sports & fitness clubs are too expensive	21	4.0	77.1
do not know how	14	2.7	79.8
thinking not need	18	3.4	83.2
other	88	16.8	100.0
Total	524	100.0	

Overweight and obesity are often measured using the BMI (Body Mass Index) scale. BMI: is a simple index commonly used to classify overweight and obesity in adults; in the study In the study calculate body mass index for all participant found 182 (34.7%) normal weight, 225(42.9%) over weight and 113 (21.6%) obese.

significant gender differences founded in the study, overweight more in female, but obesity more in the male are listed in the table (26).

Table (26) : Crosstab

Count

		range of body mass index				Total
		less than 18.5 under wt	18.5-24.9 normal wt	25-29.9 over wt	>30 obese	
Gender	male	1	44	94	61	200
	female	3	139	130	52	324
Total		4	183	224	113	524

Chi square=29.106 DF=3 p-value 0.00

Most of participants under 30 years of age are normal weight, are listed in the table (27)

Table (27) : Crosstab

Count

		range of body mass index				Total
		less than 18.5 under wt	18.5-24.9 normal wt	25-29.9 over wt	>30 obese	
Age	Under 30 years	3	104	39	15	161
	31-40 years	1	43	70	40	154
	41-50 years	0	18	67	36	121
	Over 50 years	0	18	48	22	88
Total		4	183	224	113	524

Chi-square 103.52 DF-9 p-value 0.000

No significant associated between body mass index and physical activity.

The explanation of these finding may due to types of physical activity light type than other types or do it in short time are listed in the table (28).

have physical activity * range of body mass index

Table(28) : Crosstab

Count

PHYSICAL ACTIVITY		range of body mass index				Total
		less than 18.5 under wt	18.5-24.9 normal wt	25-29.9 over wt	>30 obese	
Moderate Activity	Yes	2	54	67	31	154
	No	2	129	157	82	370
Total		4	183	224	113	524

Chi-square=1.058 DF- 3 P-value 0.787

V. Discussion, Conclusions and Recommendations

a) Discussion

This research study attempted to provide information on medical staff aged from less than 30 years to more than 50 years and their knowledge and practices of noncommunicable disease risk factors.

In a survey conducted sampling during 2015, a total of 524 female and male and their knowledge and practices towards types of physical activity, vigorous, moderate(in the leisure time, in work, also -reported the number of days of doing physical exercise per week .

Other questions about consumed fruit and vegetables (Frequencies, prevalence) and chi-square tests were conducted to detect significant or insignificant results, cross-sectional dataset on these NCD risk factor questions to answer the primary research question.

- We obtained data from 524 participants, 200 male (38.2%) & 324 female (61.8%) –
- The age distribution of participant under 30 years 161(30.7%), 31to 40 years 154(29.4%), 41 to 50 years 121(23.1%) and over 50 years 88(16.8%).
- From the total sample of 524, 106 dentists (20.2%), 81 pharmacists (15.5%), 185 general practitioners (35.3%) and 152 specialist (29%).
- About half of participant have idea about non communicable disease. Only 122(23.3) was taken lecture, workshop about it.
- Physical inactivity is a modifiable risk factor for cardiovascular disease and a widening variety of other chronic diseases, including diabetes mellitus, cancer (colon and breast), obesity, hypertension, bone and joint diseases (osteoporosis and osteoarthritis), and depression. The prevalence of physical inactivity among participant 368.2(70.2%).

Overall70.2% of the participants reported no physical exercise, high proportions of both males and females no practiced of physical activity, especially physical activity in their leisure time. However, the percentages of daily vigorous physical activity, a component of total daily.

Low proportion of females and males participated in physical activity in their leisure time. The low prevalence of leisure time physical activity is apparent throughout all age groups and no gender differences were found at the 0.05 level of significance, males reported higher percentages of daily physical activity 33% of male compared 27% in female.

Although no significant age differences were found in terms of physical activity participation showed in the table.

- 255 from 524 of participants lower dietary consumption of fruits and vegetables 48.7% concept of fruit and vegetable equal to one serving a day.
- High level of awareness about high blood pressure and diabetic disease, so percentage of measuring blood pressure, blood glucose 95.6%.
- Prevention of noncommunicable disease most of participants trying to lose weight, doing physical activity and eating healthy diet 391(74.6%) have know important normal weight.
- High percent trying to change weight 56.1%.
- In the study calculate body mass index for all participant found 182 (34.7%) normal weight, 225 (42.9%) over weight and 113 (21.6%) obese.
- Main cause don't get physical activity, no time 73.1%.
- Significant gender differences founded in the study, overweight more in female, but obesity more in the male.
- No significant associated between body mass index and physical activity .the explanation of these finding may due to types of physical activity light physical activity than other types or exercise or do it in the short time.
- Data from annual statistical report 2014 of ministry of health- Iraq.

Cerbro-vascular disease about 11%, asthma 14%, diabetes 13%, most common cancer in Iraq breast cancer 19% from total cancer, lung cancer 8.9%, colorectal cancer 5.4%.[30]

Top ten causes of death in Iraq in 2014

1. Cerebro- vascular diseases 10.08%
2. Ischemic heart disease 8.33%
3. Heart failure 7.77%
4. Renal failar 5.71%
5. Respiratory and cardiovascular disorders 5.4%
6. Hypertensive disease 5.29%
7. Mechanical exposure 5.19%
8. Diabetes mellitus 3.03%
9. Septicemia 2.43%
10. Malignant of digestive 2.13%

b) Results of other study

Higher prevalence of tobacco use and alcohol intake and a lower dietary consumption of fruits and vegetables, but physical inactivity were less frequent. Urban residence was associated with higher education, and physical inactivity. [31]

The prevalence of overweight (men – 23.9%, women – 37.5%), results showed a high burden of NCD risk factors in Kerala -India. In terms behavioral risk factors, a fifth of the sample used tobacco products, and a tenth consumed alcohol, and two-fifths consumed diet low in fruit and vegetable content (relative to some dietary guidelines), but physical inactivity was uncommon. The prevalence of smoking in men (42%) was double that observed in the United States (21%) 21, whereas that in women was quite low, consistent with cultural differences. The prevalence of a diet low in fruits and vegetables (40%) and physical inactivity, (7%) were considerably lower than in the United States where the prevalence of these behavioral habits are-70 per cent and 11-23 per cent, respectively (range of estimates for different ethnicities)(31)

Physical exercise in southern Germany Overall, 38.9% of the participants reported nonphysical exercise. Men reported a higher level of physical exercise than did women. Less exercise was reported by subjects with diabetes, high body mass index and waist-to-hip ratio and by those who were underweight. Alcohol consumption, smoker status and higher educational level showed a positive association with physical exercise.

A negative trend with respect to moderate physical exercise was observed for those with metabolic syndrome, diabetes, hypertension and hepatic statuses, but this was statistically significant only for subjects with diabetes. In both men and women, their relationship between self assessed 'good' PF and high physical exercise. [14]

- The products of tobacco, alcohol and food industries are responsible for a significant and growing proportion of the global burden of disease. Smoking and alcohol combined account for 12.5% of global deaths and 19.5% in high income countries, while six diet-related risk factors account for 13.6 and 17.5% of deaths, respectively. (32).

Except in Eastern Europe and parts of Africa, mortality among adults has declined in most countries for decades.

Lower rates of death frominfectious diseases were the early driver of this improvement, but there havebeen subsequent declines in mortality from cardiovascular disease and some cancers. 2, There have also been important trends in various cancers2 — for example, the rise and subsequent decline in lung-cancer incidence and mortality among men in many high-income countries, a decline in stomach-cancer incidence and mortality as economies develop, and the worldwide increase in breast-cancer incidence.(8) the Mongolian population aged 15-64 years old has an insufficient knowledge on the risk factors of NCDs and is not informed about benefits and options for healthy behaviors and early detection methods. In particular, knowledge about risky behaviors and health promoting and preventive behaviors is missing or insufficient as well as knowledge about self control measures, particularly in the male an young populations.

Information on CVDs, diabetes, cervical cancer, and breast cancer, and ways to prevent these diseases also showed some gaps among the population. The population lacks knowledge regarding the self control of these diseases and is not aware that by changing their own lifestyles they can influence and reduce risk factors and potentially prevent NCDs. [33]

c) Limitations

A limitation of this study is the inability to infer causality due to the cross-sectional nature of the survey.

An attempt was made to capture obesity prevalence by asking for self-reported weight and height measurements. These measurements, participant-reported age and sex, would have been used to calculate the Body Mass Index for each individual in order to assess obesity prevalence.

Despite instructions and additional clarification, recall bias may exist in the results relating to the food question on fruit and vegetable intake, and the questions regarding physical activity knowledge and practices.

d) Conclusions

Despite these limitations, this study does provide results regarding knowledge and practices towards physical inactivity and nutritional intake regarding fruits and vegetables,

- The first conclusion from this study is that participants need to improve their vigorous,

moderate activity levels to meet recommendations by the World Health Organization.

Adults aged 18–64 years

Should do at least 150 minutes of moderate-intensity physical activity throughout the week, or do at least 75 minutes of vigorous-intensity physical activity throughout the week, or an equivalent combination of moderate- and vigorous-intensity activity. [12]

- The second conclusion is that a large majority of participants are not receiving recommended daily intakes of fruits and vegetables
- Majority of participant overweight or obese, According to body mass index, percentage of overweight and obesity of the participants 42.9%, 21.6 respectively.

Most stressful cause of participants the work in spite all participants medical staff & in Iraq all medical staff work in the government aspect and sometimes work in both private & government.

References Références Referencias

1. Dr. John Everett Park. Park's Text Book of Preventive and Social Medicine. 19 th edition - India: 2009.
2. Noncommunicable diseases (world health organization) WHO; Fact sheet Updated January 2015.
3. CDC (center of disease control & prevention) 24/7: saving lives-protecting people CDC Global Noncommunicable Diseases (NCDs).
4. David J. Hunter, Sc.D., and K. Srinath Reddy. noncommunicable disease. N Engl J Med 2013; 369:1336-1343October 3, 2013 DOI: 10.1056/NEJ Mra1109345.
5. Mariachiara Di Cesare, Young-Ho Khang, Perviz Asaria, Tony Blakely, Melanie J Cowan, Farshad Farzadfa. Inequalities in non-communicable diseases and effective responses. LANCET Volume 381, No. 9866, p585–597, 16 February 2013.
6. "Non-Communicable Diseases Deemed Development Challenge of 'Epidemic Proportions' in Political Declaration Adopted During Landmark General Assembly Summit". United Nations. Department of Public Information. 19 September 2011. Retrieved 14 March 2014.
7. world health organization(WHO) ,Global Health Observatory (GHO) data. Risk factors.
8. Majid Ezzati, and Elio Riboli. Behavioral and Dietary Risk Factors for Noncommunicable Diseases. N Engl J Med 2013; 369:954-964 September 5, 2013 DOI: 10.1056/NEJMra1203528
9. Khatib O. Noncommunicable diseases: risk factors and regional strategies for prevention and care. East Mediterr Health J. 2004 Nov; 10(6): 778-88.
10. WHO | Four noncommunicable diseases, four shared risk factors http://www.who.int/ncdnet/about/4diseases/en
11. Prabhat Jha, D.Phil., and Richard Peto. -Global Effects of Smoking, of Quitting, and of Taxing Tobacco. (NJEM)N Engl J Med 2014; 370:60-68January 2, 2014DOI: 10.1056/NEJMra1308383
12. WHO Physical activity-Fact sheet N°385Updated January 2015 available http://www.who.int/topics/physical_activity/en/.
13. CDC24/7:saving Physical Activity and Health , Basics | Physical Activity | DNPAO | CDC available http://www.cdc.gov/physicalactivity/basics
14. Elli Rupps, Mark Martin Haenle, Juergen Steinacker, Richard Andrew Maso Ronald Steiner, et al. Physical exercise in southern Germany: a cross-sectional study of an urbanpopul. BMJ Open 2012; 2: e0007-13. doi:10.1136/bmjopen-2011-000713)
15. Darren E.R. Warburton, Crystal Whitney Nicol, and Shannon S.D. Bredin. Health benefits of physical activity: the evidence. CMAJ. 2006 Mar 14; 174(6): 801–809.
16. Arem H, Moore SC, Patel A, Hartge P, Berrington de Gonzalez A, Visvanathan K, et al .Leisure time physical activity and mortality: a detailed pooled analysis of the dose-response relationship. - JAMA Intern Med. 2015 Jun; 175(6): 959-67. doi: 10.1001/jamainternmed.2015.0533
17. Burzynska AZ, Wong CN, Voss MW, Cooke GE, Gothe NP, Fanning J, McAuley E, Kramer AF.. Moment-To-Moment Variability in Spontaneous Brain Activity in Older Adults. PLoS One. 2015 Aug 5; 10(8): e0134819. doi: 10.1371/journal.pone.013 4819.
18. Centers for disease control and prevention. CDC24/7: SAVING LIVES, PROTECTING PEOPLE available. http://www.cdc.gov/physicalactivity/basics/adults
19. WHO | Diet(http://www.who.int/topics/diet)
20. WHO -Healthy diet Fact sheet N°394Updated September 2015
21. Na L, Han T, Zhang W, Wu X, Na G, Du S, Li Y, et al. A Snack Dietary Pattern Increases the Risk of Hypercholesterolemia in Northern Chinese Adults: A Prospective Cohort Study. PLoS One. 2015 Aug 5; 10(8): e0134294. doi: 10.1371/journal.pone.0134 294. eCollection 2015
22. WHO | Alcohol - World Health Organization www.who.int/topics/alcohol-dring/en
23. WHO | Alcohol - World Health Organization , Alcohol, Fact sheet Updated January 2015
24. CDC - Fact Sheets-Alcohol Use And Health - Alcohol http://www.cdc.gov/alcohol/fact-sheets/alcohol-use.htm
25. WHO | Obesity http://www.who.int/topics/obesity/en/
26. Laura Webber, Diana Divajeva, Tim Marsh,Klim McPherson, Martin Brown, Gauden Galea. The future burden of obesity-related diseases in the 53 WHO European-Region countries and the impact of

effective interventions: a modelling study: BMJ Open 2014;4:e004787 doi:10.1136/bmjopen-2014-004787
27. Danaei G1, Vander Hoorn S, Lopez AD, Murray CJ, Ezzati M, Comparative Risk Assessment collaborating group (Cancers). Causes of cancer in the world: comparative risk assessment of nine behavioral and environmental risk factors. Lancet. 2005 Nov 19; 366(9499):1784-93.
28. Anna B. Gilmore, Emily Savell and Jeff Collin. Public health, corporations and the New Responsibility Deal: promoting partnerships with vectors of disease. Oxford Journals, Medicine & Health. Journal of Public Health Volume 33, Issue 1 Pp. 2-4
29. Lixin Na, Tianshu Han, Wei Zhang, Xiaoyan Wu, Guanqiong Na, Shanshan Du, et al. A Snack Dietary Pattern Increases the Risk of Hypercholesterolemia in Northern Chinese Adults: A Prospective Cohort Study. PLoS One. 2015; 10(8): e0134294. Published online 2015 Aug 5.
30. Annual statistical report 2014 (ministry of health-Iraq)
31. K.R. Thankappan, Bela Shah*, Prashant Mathur*, P.S. Sarma, G. Srinivas**, G.K. Mini, et al. Risk factor profile for chronic non-communicable diseases: Results of a community-based study in Kerala, India. Indian J Med Res 131, January 2010, pp 53-63.
32. Department for Health, University of Bath, Bath BA2 7AY, UK.2European Centre for Health of Societies in Transition, London, School of Hygiene and Tropical Medicine,London WC1E 7HT,UK3UK Centre for Tobacco Control Studies, Nottingham NG5 1PB,UK 4Global Public Health Unit, University of Edinburgh, Edinburgh. Editorial. Journal of Public Health | Vol. 33, No. 1, pp. doi:10.1093/pubmed/fdr008 | Advance Access Publication 2 February 2011.
33. SURVEY REPORT, -Knowledge, Attitudes and Practices related to the Non-communicable Diseases among Mongolian General Population-2010 PARTICIPATING ORGANIZATIONS: MCA Mongolia Health project EPOS Health Management.

This page is intentionally left blank

Global Journal of Medical Research: F
Diseases
Volume 16 Issue 3 Version 1.0 Year 2016
Type: Double Blind Peer Reviewed International Research Journal
Publisher: Global Journals Inc. (USA)
Online ISSN: 2249-4618 & Print ISSN: 0975-5888

Systemic and Local Immune Response to H. Pylori Infection and their Correlation with the Degree of Antral and Duodenal Inflammation

By Awad Magbri & F. Stevens
University College Hospital Galway, United States

Abstract- *Design, setting, participants & measurements:* One hundred and eight patients presented to the University College Hospital Galway, Ireland (UCHG) with upper gastro-intestinal symptoms were included in the study. There were 52 males with age range 18-82 years, mean age 49 years, and 56 females, age range 20-83 years, mean age 52 years. They were grouped according to the endoscopic findings into 4 groups (i) Duodenal Ulcer (DU), (ii) Gastric Ulcer (GU), (iii) Gastritis (GS), & (iv) Non-Ulcer Dyspepsia (NUD). Five milliliter of venous blood was taken in a sterile plain tube; the serum was separated and stored at -70 C0 for ELISA assay. Endoscopy was carried out and at least 4 biopsies were obtained, 2 from the antrum and 2 from the first part of the duodenum (duodenal bulb). One biopsy each from the antrum and duodenum was transferred immediately into a sterile container containing 2 mL of RPMI 1640. The other biopsy specimens one from the antrum and one from the duodenum were processed for histological examination. The serum IgG antibodies to H. pylori and local IgA and IgG were measured by an ELISA test.

GJMR-F Classification : *NLMC Code: WD 308*

Strictly as per the compliance and regulations of:

Systemic and Local Immune Response to H. Pylori Infection and their Correlation with the Degree of Antral and Duodenal Inflammation

Awad Magbri [α] & F. Stevens [σ]

Abstract- Design, setting, participants & measurements: One hundred and eight patients presented to the University College Hospital Galway, Ireland (UCHG) with upper gastro-intestinal symptoms were included in the study. There were 52 males with age range 18-82 years, mean age 49 years, and 56 females, age range 20-83 years, mean age 52 years. They were grouped according to the endoscopic findings into 4 groups (i) Duodenal Ulcer (DU), (ii) Gastric Ulcer (GU), (iii) Gastritis (GS), & (iv) Non-Ulcer Dyspepsia (NUD). Five milliliter of venous blood was taken in a sterile plain tube; the serum was separated and stored at -70 C^0 for ELISA assay. Endoscopy was carried out and at least 4 biopsies were obtained, 2 from the antrum and 2 from the first part of the duodenum (duodenal bulb). One biopsy each from the antrum and duodenum was transferred immediately into a sterile container containing 2 mL of RPMI 1640. The other biopsy specimens one from the antrum and one from the duodenum were processed for histological examination. The serum IgG antibodies to H. pylori and local IgA and IgG were measured by an ELISA test. Serum IgG antibody levels of the four groups of patients with various degrees of inflammation (0-3 degree) were compared, a highly significant association was found between the level of IgG antibodies to H. pylori and the severity of inflammatory reaction in the biopsy specimens (0 &2 and 0 & 3, p=0.0001) and between grade (1 & 2, p=0.045) and (1 &3, p=0.002) but not between (0&1, p=0.310), and (2&3, p=0.282) grades of inflammation.

Duodenal and antral IgA and IgG antibodies to H. pylori were assessed in 108 patients. A higher number of antral compared to duodenal biopsies in culture produce both IgA and IgG antibodies to H. pylori in all groups. 21/25 (84%) of DU patients had IgG antibodies to H. pylori, 22/25 (88%) had antral IgA antibodies, 19/25(76%) had antral IgG antibodies, 12/25 (48%) had duodenal IgA antibodies and 7/25(28%) had duodenal IgG antibodies to H. pylori.

In GU group 6/6(100%) patients had serum IgG antibodies to H. pylori, only 4/6(66.7%) had antral IgA antibodies, and 3/5(60%) had antral IgG antibodies. Duodenal antibodies were much less common than in the DU group, only 1/5(20%) had duodenal IgA antibodies and 0/5(0.0%) had IgG antibodies to H. pylori infection. In GSs group 20/31(65%) had serum IgG antibodies to H. pylori. 19/31(61.3%) had IgA antibodies, 13/28(46.43%) had antral IgG antibodies, 4/29 (13.8%) had duodenal IgA antibodies and 3/29(10.34%) had duodenal IgG antibodies to H. pylori. In the NUD group 23/46 (50%) had serum IgG antibodies to H. pylori, 27/46 (58.7%) had antral IgA antibodies, 17/43(39.5) had antral IgG antibodies, 8/44(18.2%) had duodenal IgA antibodies and 7/44(15.9%) of patients had duodenal IgG antibodies to H. pylori. The level of serum IgG to H. pylori was significantly different between the GS and NUD (p=0.013).

Conclusion: H. pylori is capable of inflecting local and systemic immune response with production of local (antral and duodenal) IgA and IgG and systemic IgG that correlate with the degree of inflammation in the antrum and duodenum. Successful treatment of the infection with antibiotics and proton pump inhibitors may influence the degree of inflammation, the local and systemic immune response to the organisms.

I. Introduction

Helicobacter pylori are now recognized as a primary cause of active chronic gastritis in humans. Most infected humans remain asymptomatic, but are at increased risk for developing peptic ulcer disease and possibly gastric cancer (1-8). The pathogenesis of this infection is well understood and the motility and urease activities are virulent factors in an animal model (9, 10). The urease enzyme also elicits a strong immune response during acute infection, suggesting that this abundant antigen is readily available to the immune system. An increase in serum IgG titers is predictive of ongoing infection (11, 12). About 50% of H. pylori isolates produce vacuolating toxins in vitro, which may be an important determinant of virulence (12-15). The epidemiology of the infection by H. pylori correlates with the prevalence of superficial type B gastritis. Infection is associated with active (presence of neutrophils), chronic (presence of lymphocytes and plasma cells), or active-chronic (neutrophils, lymphocytes, and plasma cells) inflammatory reactions (16-18). Gastric inflammation nearly always precedes the development of peptic ulceration, and is an important component in initiating the multi-step progression towards gastric carcinogenesis (19). In this study we have demonstrated that the severity of inflammation in the antrum is strongly associated with the presence and density of the bacterial colonization of the antral mucosa and the level of the serum IgG antibody to H. pylori.

II. Subjects and Methods

One hundred and eight patients presented to the University College Hospital Galway, Ireland (UCHG)

Author α: Dialysis Access Center of Pittsburgh, PA.
e-mail: elmagbri@hotmail.com
Author σ: University College Hospital Galway, Ireland.

with upper gastro-intestinal symptoms were included in the study. There were 52 males with age range 18-82 years, mean age 49 years, and 56 females, age range 20-83 years, mean age 52 years. They were grouped into 4 groups according to the endoscopic findings; (i) Duodenal Ulcer group (DU), (ii) Gastric Ulcer group (GU), (iii) Gastritis group (GS), and (iv) Non-Ulcer Dyspepsia group (NUD).

Endoscopy was carried out and at least 4 biopsies were obtained, 2 from the antrum and 2 from the first part of the duodenum (duodenal bulb). One biopsy each from the antrum and duodenum was transferred immediately into a sterile container containing 2 mL of RPMI 1640 (Gibco Life Technology Ltd. UK) supplemented with 10% fetal calve serum (FCS) and 40 ug/ml gentamicin (sigma). The biopsy specimens were cultured for 3 days at $37C^0$; the unspun supernatants were removed and stored at $-70C^0$ for estimation of IgA and IgG antibodies against H. pylori (34). The other biopsy specimens one from the antrum and one from the duodenum were placed in formalin and processed for histological examination. Five milliliter of venous blood was taken in a sterile plain tube; the serum was separated and stored at -70 C^0 for ELISA assay.

The serum IgG antibodies to H. pylori were measured by a commercially available ELISA kits (Biometra, Germany). Local IgA and IgG antibodies to H. pylori in the supernatants were measured by ELISA kit (KPL Kierkegaard & Perry Laboratories, Inc.) and the results were read on Dynatech MR 5000 automatic micro-plate reader at wave length 410 nm.

Endoscopy: all endoscopies were performed by two experienced endoscopists (CLL and SFM). Endoscopic findings were registered and stored in a computer data base. Biopsy specimens for histological examination, culture of the organisms, and estimation of local antibodies to H. pylori in the cultured biopsy materials were taken from the antrum and duodenal bulb. The endoscopes (Olympus Gastrscope type GIF-Q10 and GIF-Q20) and biopsy forceps were cleaned and disinfected in a commercially available 2% glutaraldehyde solution (Totacide 28 Coventry Chemicals Ltd) for a period of 10 minutes between each endoscopy.

a) Estimation of local and systemic H. pylori antibodies

ELISA assay for estimation of systemic antibodies: H. pylori antibody test is an enzyme linked immune-sorbent assay (ELISA) for the quantitative determination of H. pylori IgG antibodies in human serum or plasma. The methodology is based on using the solid phase technique on the micro-wells. The purified H. pylori specific antigens are immobilized into the surface of the micro-wells. The antigens react with H. pylori IgG antibodies present in both the standards and patient samples. The resulting antigen-antibody complex binds the second enzyme-conjugate antihuman IgG antibody, creating a sandwich. The enzyme conjugated antigen-antibody complex reacts with added substrate to develop a colored solution. The intensity of the color is dependent on the amount of the enzyme coupled to the complex and proportional to the H. pylori antibody concentration in the patient's samples. The optical density (OD) of the color in each well is read using Dynatech MR 5000 automatic micro-plate reader at 450 nm. Serum IgG antibodies to H. pylori were measured using the computer constructed formula for the semi-log curve fit. $Y = a + b\ Logx$

Where (Y) is the mean of the OD of the sample, (a)-is the Y intercept of the graph and (b) is the constant factor for (Logx). Having obtained the value of Logx from the equation the inversion of that value represents the amount of the IgG in the sample expressed in ELISA units.ml sample (E.U/ml sample). The assay procedure is done at room temperature ($18\text{-}22C^0$), higher temp up to $35C^0$ does not interfere with the results of the test but the incubation time for the TMB enzyme substrate reaction should be reduced according to the specifications of the manufacturer.

ELISA assays for estimation of local antibodies: Local IgA and IgG antibodies in the supernatants of the cultures biopsy materials from the duodenal bulb and the antrum were assayed using the ELISA assay as specified by the manufacture (KPL Kierkegaard & Perry Lab Inc.)

b) Statistical methods

The analysis of the results of the serum IgG antibody levels to H. pylori were assessed using One Way Analysis of Variance (ANOVA) with the addition of the Tukey HSD test option to the statistics. The additions of the Tukey option to the statistic command in the one-way analysis of variance will protect from declaring pairs of means differ when they could differ by chance. Analysis of variance offers a standard method for comparing various groups when there is no presumption beforehand that they differ. The Tukey's method exemplifies those tests for difference among group means by using the difference between the largest and smallest means, often called the range, as a measure of their dispersion. We have used the SYSTAT computer package (1990, SYSTAT inc.) for ANOVA with Tukey's option. Chi square was used to analyze the difference of the local and systemic humoral immune response to H. pylori infection. Pearson's correlation method was used for statistical analysis of the relationship between the density of H. pylori and serum IgG antibody levels.

III. Results

a) Antral inflammation Vs serum IgG antibodies to H. pylori

When the serum IgG antibody levels of the four groups of patients with various degrees of inflammation

(0-3 degree) were compared, a highly significant association was found between the level of IgG antibodies to H. pylori and the severity of inflammatory reaction in the biopsy specimens. One was analysis of variance with Tukey HSD multiple comparisons was applied for statistical analysis. There were a significant difference of serum IgG antibody levels to H. pylori between grade (0 &2 and 0 & 3, p=0.0001) and between grade (1 & 2, p=0.045) and (1 &3, p=0.002) but not between (0&1, p=0.310), and (2&3, p=0.282) grades of inflammation, Table-1.

b) Local and systemic humoral immune response to H. pylori

In vitro production of duodenal and antral IgA and IgG antibodies to H. pylori were assessed in 108 patients with dyspepsia. There were divided into four subgroups according to the endoscopic findings (i) DU group (25 patients), (ii) GU group (6 patents), (iii) GS group (31 patients), (iv) NUD group (56 patients), Tables -2-6. The results were analyzed using the Chi square test and the p values were shown in Table -6.

A higher number of antral compared to duodenal biopsies in culture produce both IgA and IgG antibodies to H. pylori in all groups (tables 2-6). This is an expected finding as H. pylori only colonize duodenum if gastric metaplasia is present. 21/25 (84%) of DU patients had IgG antibodies to H. pylori, 22/25 (88%) had antral IgA antibodies, 19/25(76%) had antral IgG antibodies, 12/25(48%) had duodenal IgA antibodies and 7/25(28%) had duodenal IgG antibodies to H. pylori, Table 2.

In the GU group although 6/6(100%) patients had serum IgG antibodies to H. pylori, only 4/6(66.7%) had antral IgA antibodies, and 3/5(60%) had antral IgG antibodies. Duodenal antibodies were much less common than in the DU group, only 1/5(20%) had duodenal IgA antibodies and 0/5(0.0%) had IgG antibodies to H. pylori infection, Table 3.

In the GS group 20/31(65%) had serum IgG antibodies to H. pylori. 19/31(61.3%) had IgA antibodies, 13/28(46.43%) had antral IgG antibodies, 4/29(13.8%) had duodenal IgA antibodies and 3/29(10.34%) had duodenal IgG antibodies to H. pylori, Table 4.

In the NUD group 23/46(50%) had serum IgG antibodies to H. pylori, 27/46(58.7%) had antral IgA antibodies, 17/43(39.5) had antral IgG antibodies, 8/44(18.2%) had duodenal IgA antibodies and 7/44(15.9%) of patients had duodenal IgG antibodies to H. pylori, Table 5.

The difference between the local (antral and duodenal) IgA and IgG antibodies in the DU, GU, GS, and NUD groups are shown in Tables 2-6.

The level of serum IgG to H. pylori was significantly different among the 4 groups when tested by the Kruskal-Wallis One Way Analysis of variance (p=0.0008). However, when the multiple comparisons using the Tukey HSD One way analysis of variance was applied the significant difference was found to be residing in the GS and NUD groups (p=0.013). There were no significant difference between the other groups.

IV. Discussion

Chronic gastritis with a prominence of lymphocytes and plasma cells is considered to be a morphologic indicator of an immune response to H. pylori infection (175). Indeed, a local immune response and production of antibodies against H. pylori have been demonstrated in patients with gastritis by Grabtree and others (19-21) and have been confirmed in this study also (Tables 2-6). Berstad et al (22) have demonstrated that the characteristics (severity and cell type) of gastritis associated with H. pylori infection are influenced by geographical factors that may be similar to those that modify infection rates for different geographical locations. The pathogenesis of the bacterium depends on the production of several virulence factors. The most important ones are CagA (cytotoxic associated gene A) and VacA (vacuolizing cytotoxin A) (23-27). We have found a relatively good correlation between the serology and the histological findings in antral biopsies despite the low sensitivity (63%) of our ELISA test compared to histology and culture. Our results agreed with Bertram et al (28, 29) that atrophic gastritis was associated with low detection rate of H. pylori by histology.

The reliability of culture of H. pylori as a diagnostic test varies considerably from center to center. Many workers found a sensitivity of 85% as we did. Our results showed lower sensitivity and specificity (77.4% and 75%) of histology. ELISA assay has been widely utilized in the sero-epidemiological studies and in the follow up of treated patients with H. pylori infection (30). We recommended determining IG antibody as a pre-endoscopic screening test. Further improvement and refinement of H. pylori antigens and proper exploitation of this test may have a great implication on the work load of busy endoscopic units and on the economy of health services. In H. pylori both IgA and IgG antibody titers are significantly elevated. On the other hand, the IgM antibodies are similar in H. pylori positive and negative subjects (31, 32). Probably because the IgM sero-conversion in the early phase of infection is rapidly transit and usually will not be detected. Acid-glycin extraction and more purified antigens to H. pylori raise the sensitivity and specificity of ELISA to H. pylori to 95-96% and 74-83% respectively (33-37). Although the antigen we used for ELISA assay was purified antigen, our ELISA lacks a sufficient sensitivity (63%, but has a high specificity (97%) for H. pylori.

Talley et al (38) evaluated the sensitivity and specificity of different ELISA and found them with high

sensitivity and specificity 96 and 94% respectively. Newell et al (39) also reported high sensitivity and specificity of ELISA (100%) in 47 patients who had endoscopy and confirmed by histology and culture.

Prieto et al (40) found that nodular anteritis was a frequent (67%) and a specific finding in H. pylori infection. Their presence was associated with lymphoid follicles in histopathological examination. Our results agreed with Prieto et al. Moderate inflammation were present in 9/25(36%) of our patients, all were H. pylori positive. We concluded that the majority of the elderly (>61 yrs) had a prevalence of H. pylori ranging from 45-64% depending on the mode used to detect H. pylori infection. More than 50% of this group had only mild inflammation and H. pylori was isolated in 43%, however, moderate to severe degree of inflammation was present in 44% of them and H. pylori was always associated with gastritis. If we interpret this association in the light of serum IgG antibodies to H. pylori we will find that 95% of the elderly people with moderate to severe degree of inflammation will have an elevated serum IgG antibody levels of 220-250 EU for moderate inflammation and >250 EU for severe inflammation.

The evolving knowledge of the immune reaction to H. pylori infection allows us to study the inter-relationship between (i) the presence of H. pylori and the density of the organisms on the histological section from the antral biopsies, (ii) the severity of inflammation, and (iii) the serum IgG antibodies to H. pylori as a marker of established infection. The presence of the bacteria was associated with increased amounts of mononuclear inflammatory cells, neutrophilic and eosinophilic leukocytes in the antral biopsies, thus confirming the results of Karttunen et al (41) and Alam et al (42). In these patients the infection with H. pylori produces a higher number of local IgA and IgG antibodies to H. pylori in the antral compared to duodenal biopsies in culture, in all groups (p=0.0296), Tables 2-6.

V. Conclusion

Helicobacter pylori are now recognized as a primary cause of active chronic gastritis in humans. Most infected humans remain asymptomatic, but are at increased risk for developing peptic ulcer disease and possibly gastric cancer. H. pylori is capable of inflecting local and systemic immune response with production of local (antral and duodenal) IgA and IgG and systemic IgG that correlate with the degree of inflammation in the antrum and duodenum. Successful treatment of the infection with antibiotics and proton pump inhibitors may influence the degree of inflammation, the local and systemic immune response to the organisms.

References Références Referencias

1. Weck MN, Gao L, Brenner H. Helicobacter pylori infection and chronic atrophic gastritis: associations according to severity of disease. Epidemiology. 2009; 20(4): 569-574
2. Dixon MF, Genta RM, Yardley JH, et al. Classification and grading of gastritis. The update Sydney System. International Workshop on the Histopathology of Gastritis, Houston 1994. Am J Surg Pathol. 1996; 20(10): 1161-1181
3. Blaser MJ, Atherton JC. Helicobacter pylori persistence: biology and disease. J Clin Invest. 2004; 113(3): 321-333
4. Ciociola AA, McSorley DJ, Turner K, et al. Helicobacter pylori infection rates in duodenal ulcer patients in the United States may be lower than previously estimated. Am J Gastroenterol 1999; 94: 1834
5. Parsonnet J, Friedman GD, Vandersteen DP, et al. Helicobacter pylori infection and gastric lymphoma. N Eng J Med. 1994; 330: 1267-1271
6. Wen S, Moss SF. Helicobacter pylori virulence factors in gastric carcinogenesis. Cancer Letters. 2009; 282(1): 1-8
7. Kusters JG, van Vliet AH, Kuipers EJ. Pathogenesis of Helicobacter pylori infection. Clinical Microbiology Reviews 2006; 19(3): 449-490
8. Sagaert X, Van Cutsem E, De Hertogh G, et al. Gastric MALT lymphoma: A model of chronic inflammation-induced tumor development. Nature Reviews Gastroenterology & Hepatology 2010; 7(6): 336-346
9. Blaser MJ. Hypotheses in the pathogenesis and natural history of Helicobacter pylori induced inflammation. Gastroenterology. 1992; 102: 720-727
10. Smooth DT, Mobley HL, Chippendale GR, et al. Helicobacter pylori urease activity is toxic to human gastric epithelial cells. Infec. Immuno. 1990; 58(6): 1992-1994
11. Mobley HLT, Hu LT, Foxall PA. Helicobacter pylori: properties and role in pathogenesis. Scand J Gastroentrol. 1991; 26(suppl 187): 39-46
12. Newell DG. Virulence factors of Helicobacter pylori. Scand J Gastroentrol. 1991; 26(suppl. 187): 31-38
13. Cover TL, Vaughn SG, Cao P, et al. Potentiation of Helicobacter pylori vacuolating toxin activity by nicotin and other weak bases. J Infect Disease 1992; 166: 1072-1078
14. Cover TL, Dooley CP, Blaser MJ. Characterization and human serology response to proteins in Helicobacter pylori broth culture supernatants with vacuolzing cytotoxin activity. Infec. Immu. 1990; 58(3): 603-610
15. Heatley RV. Helicobacter pylori infection and inflammation. Scand J Gastroenterol 1991; 26(suppl 187): 23-30
16. Kartunen T, Niemela S, Lehtola J. Helicobacter pylori in dyspeptic patients: quantitative association with severty of gastritis, intragastric pH, and serum

gastrin concentration. Scand J Gastroentrol. 1991;)suppl 186): 124-134
17. Mollenkoph C, Steininger H, Weineck G, Meyer M. gastritis: immunohistochemical detection of specific and nonspecific immune response to Helicobacter pylori. Zeitschrift Fur Gastroenterol. 1990; 28(7): 327-34
18. Prieto G, Polanco I, Larrauri J, et al. Helicobacter pylori infection in children: clinical, endoscopic, and histologic correlations. J Paed gastroentrol & Nutrit. 1992; 14(4): 420-425
19. Crabtree JE, Shallcross TM, Wyatt JI, et al. Mucosal humoral immune response to Helicobacter pylori in patients with duodenitis. Dig Dis Scien. 1991; 36(9): 1266-1273
20. Stromberg E, Edebo A, Svennerholm AM, et al. Decreased epithelial cytokine responses in the duodenal mucosa of Helicobacter pylori-infection duodenal ulcer patients. Clin Diagn Lab Immunol. 2003; 10: 116-124
21. Hamlet A, Thoreson AC, Nilsson O, et al. Duodenal Helicobacter pylori infection differs in cagA genotype between asymptomatic subjects and patients with duodenal ulcers. Gastroenterology 1999; 116: 259-268
22. Berstad AE, Holbjorn K, Bukholm G, et al. Complement activation directly induced by Helicobacter pylori. Gastroenterology 2001; 120: 1108-1116
23. Mosbley HL. Defining Helicobacter pylori as a pathogen: strain heterogeneity and virulence. Am. J Med. 1996; 100; 2S-9S, discussion 9S-11S
24. Rohde M, Puls J, Buhrdorf R, et al. A novel sheated surface organelle of Helicobacter pylori cag type IV secreation system. Moecular Microbiology 2003; 49(1): 219-234
25. Backert S, Selbach M, Role of type IV secretion in Helicobacter pylori pathogenesis. Cellular Microbiology 2008; 10(8): 1573-1581.
26. Odenbreit S, Puls J, Sedlmaier B, et al. translocation of Helicobacter pylori CagA into gastric epithelial cells by IV secretion. Science 2000; 287(5457): 1497-1500
27. Higashi H, Tsutsumi R, Muto S, et al. SHP-2 tyrosine phosphatase as na intracellular target of Helicobacter pylori Cag A protein. Science 2002; 295: 683-686
28. Bertram TA, Murray PD, Morgan DR, et al. Gastritis associated with infection by Helicobacter pylori in humans: Geographical differences. Scand J Gastroentrol. 1991; 26(suppl 181): 1-8
29. Klein PD, Graham DY, Gaillour A, et al. Water sources as risk factor for Helicobacter pylori infection in Peruvian children. Lancet. 1991; 337(8756): 1503-1506
30. Kosunen TU, Seppala K, Sarna S, Sipponen P. Diagnostic value of decreasing IgG, IgA, and IgM antibody titers after eradication of Helicobacter pylori. The Lancet. 1992; 339: 893-895
31. Maddocks AC. Helicobacter pylori (formerly campylobacter pylori) 1986-1989: A review. J Clin Pathol. 1990; 43: 353-356
32. Rauws EAJ, Tytgat GNJ. Campylobacter pylori. Gist-brocades Pharmaceuticals. Division of Royal Gist-brocades NV, Delft, The Netherlands. 1989.
33. Dent JC, McNullty CAM, Uff JS. Et al. Campylobacter pylori urease: a new serological test. Lancet. 1988; 1002
34. Goodwin CS, Blincow E, Peterson G, et al. Enzyme-Linked Immunosorbent Assay for Campylobacter pyloridis: Correlation with presence of C. pyloridis in the Gastric Mucosa. J Infect Dis 1987; 155(3): 488-494
35. Newell DG. An Enzyme-Linked Immunosorbent Assay for the serodiagnosis of Campylobacter pylori-associated gastritis. Scand J Gastroenterol. 1988; 23(suppl 142): 53-57
36. Rathbone BJ, Wyatt JI, Worsley BW, et al. Immune response to Campylobacter pyloridis. Lancet. 1985; 25: 1217
37. Steer HW, Hawtin PR, Newell DG. An ELISA technique for the serodiagnosis of Campylobacter pyloridis infection in patients with gastric and benign duodenal ulceration. Serodiag Immunother. 1987; 1: 253-259
38. Talley NJ, Newell DG, Ormand JE, et al. Serodiagnosis of Helicobacter pylori: comparison of enzyme-linked Immunosorbent assays. J Clinical Microbiol. 1991; 29(8): 1635-1639
39. Newell DG, Hawtin PR, Stacey AR, et al. Estimation of prevalence of Helicobacter pylori infection in an asymptoatic elderly population comparing [14C] urea breath test and serology. J Clin Pathology. 1991; 44(5): 385-387
40. Prieto G, Polanco I, Larrauri J, et al. Helicobacter pylori infection in children: endoscopic, and histology correlations. J Paed Gastroenterol & Nutri. 1992; 14(4): 420-425
41. Karttunen T, Niemela S, Lehtola J. Helicobacter pylori in dyspeptic patients: quantitative association with severity of gastritis, intragastric pH, and serum gastrin concentration. Scand J gastroenterol. 1991; (suppl 186): 124-134
42. Alam K, Schubert TT, Bologna SD, Ma CK. Increased density of Helicobacter pylori on antral biopsy is associated with severity of acute and chronic inflammation and likelihood of duodenal ulceration. Amer J Gastroenterol. 1992; 87(4): 424-428

Table- 1 : the severity of inflammation Vs serum H, pylori IgG antibody levels Matrix of pairwise absolute mean difference

	0	1	2	3
0	0.000			
1	24.239	0.000		
2	59.366	35.128	0.000	
3	92.460	98.221	33.094	0.000

Tukey HSD multiple comparisons

Matrix of pairwise comparison probabilities

	0	1	2	3
0	1.000			
1	0.310	1.000		
2	0.0001*	0.045*	1.000	
3	0.0001*	0.002*	0.282	1.000

**Significant results*

Table - 2 : Systemic (serum IgG antibody) Vs local (antral and duodenal IgA and IgG antibodies) to H. pylori in D>U patients

	Serum IgG	Antral IgA	Antral IgG	Duodenal IgA	Duodenal IgG
# of patients	25	25	25	25	25
Positive	21	22	19	12	7
negative	4	3	6	13	18
% positive	84%	88%	76%	48%	28%

Table - 3 : Systemic (serum IgG antibody) Vs local (antral and duodenal IgA and IgG antibodies) to H. pylori in G.U patients

	Serum IgG	Antral IgA	Antral IgG	Duodenal IgA	Duodenal IgG
# of patients	6	6	5	5	5
Positive	6	4	3	1	0
Negative	0	2	2	4	5
% positive	100%	66.7%	60%	20%	0%

Table - 4 : Systemic (serum IgG antibody) Vs local (antral and duodenal IgA and IgG antibodies) to H. pylori in gastritis patients

	Serum IgG	Antral IgA	Antral IgG	Duodenal IgA	Duodenal IgG
# of patients	31	31	28	29	29
positive	20	19	13	4	3
Negative	11	12	15	25	26
% positive	65%	61.3%	46.43%	13.8%	10.34%

Table - 5 : Systemic (serum IgG antibody) Vs local (antral & duodenal IgA & IgG antibodies) to H. pylori in patients with NUD

	Serum IgG	Antral IgA	Antral IgG	Duodenal IgA	Duodenal IgG
# of patients	46	46	43	44	44
Positive	23	27	17	8	7
Negative	23	19	26	36	37
% positive	50%	58.7%	39.5%	18.2%	15.9%

Table - 6 : The p value for the difference between positive and negative IgA and IgG antibodies to H. pylori in all groups

	DU	GU	GS	NUD
IgA antibody	0.0064*	0.35	0.0044*	0.0002*
IgG antibody	0.0018*	0.17	0.0062*	0.026*

NUD – non-ulcer dyspepsia

**significant result*

The overall probability is 0.0296 for the IgA and IgG status in all groups.

Global Journal of Medical Research: F
Diseases
Volume 16 Issue 3 Version 1.0 Year 2016
Type: Double Blind Peer Reviewed International Research Journal
Publisher: Global Journals Inc. (USA)
Online ISSN: 2249-4618 & Print ISSN: 0975-5888

Oncolytic Activity of Bacteria used in Cancerous Disease Gene Therapy

By Valencakova, A., Dziakova, A. & Hatalova, E.
University of Veterinary Medicine and Pharmacy

Abstract- Gene therapy is a therapeutic strategy based on using genes as pharmaceuticals. Gene therapy holds promise for treating a wide range of diseases, including cancer, cystic fibrosis, heart disease, diabetes, hemophilia and AIDS. Various types of genetic material are used in gene therapy; double-strained DNA (dsDNA), single strained DNA (ssDNA), plasmid DNA and antisense oligodeoxynucleotides (ASON), adenoviruses, retroviruses, undeveloped/ plasmid DNA and bacteria. The use of bacteria in cancer therapy can be advantageous for various reasons compared to classic chemotherapy or other microorganisms. Bacteria can adhere and invade tumor cells, and they are capable of proliferation and of establishing extracellular colonies. Other than that, their genome length enables them to be recipient to a quantum of exogenous therapeutic genes (for example, enzymes activating precursors and cytokines). The most important thing from the clinical safety view is they can be killed by antibiotics (such as metronidazole) if complications in further treatment arise. For comparison, the capacity of viral vectors is limited and in case of side effects viruses cannot be eliminated by antibiotics.

Keywords: *gene therapy, salmonella spp., clostridium spp., therapeutic strategy.*

GJMR-F Classification : *NLMC Code: QW 167*

Strictly as per the compliance and regulations of:

Oncolytic Activity of Bacteria used in Cancerous Disease Gene Therapy

Valencakova, A [α]., Dziakova, A [σ]. & Hatalova, E [ρ].

Abstract- Gene therapy is a therapeutic strategy based on using genes as pharmaceuticals. Gene therapy holds promise for treating a wide range of diseases, including cancer, cystic fibrosis, heart disease, diabetes, hemophilia and AIDS. Various types of genetic material are used in gene therapy; double-strained DNA (dsDNA), single strained DNA (ssDNA), plasmid DNA and antisense oligodeoxynucleotides (ASON), adenoviruses, retroviruses, undeveloped/plasmid DNA and bacteria. The use of bacteria in cancer therapy can be advantageous for various reasons compared to classic chemotherapy or other microorganisms. Bacteria can adhere and invade tumor cells, and they are capable of proliferation and of establishing extracellular colonies. Other than that, their genome length enables them to be recipient to a quantum of exogenous therapeutic genes (for example, enzymes activating precursors and cytokines). The most important thing from the clinical safety view is they can be killed by antibiotics (such as metronidazole) if complications in further treatment arise. For comparison, the capacity of viral vectors is limited and in case of side effects viruses cannot be eliminated by antibiotics.

Keywords: gene therapy, salmonella spp., clostridium spp., therapeutic strategy.

I. Introduction

The use of bacteria in cancer therapy can be advantageous for various reasons compared to classic chemotherapy or other microorganisms, such as vectors on the basis of viruses used in gene therapy. Several bacterial species are motile and have the capability of active movement against the diffuse gradient pressure built up in the abnormal environment of a tumor. On the other hand, small molecules of medicaments or viruses are dependent on streaming for them to disseminate in the tumor. For this reason, interstitial pressure in tumors limits their penetration (1). Bacteria can adhere and invade tumor cells, and they are capable of proliferation and of establishing extracellular colonies. Other than that, their genome length enables them to be recipient to a quantum of exogenous therapeutic genes (for example, enzymes activating precursors and cytokines). The most important thing from the clinical safety view is they can be killed by antibiotics (such as metronidazole) if complications in further treatment arise. For comparison, the capacity of viral vectors is limited and in case of side effects viruses cannot be eliminated by antibiotics (2).

Clostridia: several studies from half of the 20th century (3) shown that Gram-positive anaerobic *Clostridia* can proliferate in hypoxic or necrotic tissues in tumor regions and so oncolytic means for cancer treatment were proved. *Clostridia* are spore-forming anaerobic bacteria which must be injected to a patient in the form of spores. These spores migrate to the localization of the tumor and are capable of budding only in anoxic environment (note: this type of environment is present in large tumors; 2).

One of the first strains tested as an anti-cancer agent is *Clostridium histolyticum*. A direct injection of spores to mice sarcomas induced a visible tumor regression and lysis. Simultaneous microscopic examination of these bacteria proliferating inside a tumor revealed a presence of an extremely virulent strain of *Clostridium tetani*a few years later. Despite their ability to diminish tumors, these species invoked high toxicity after injection, causing quick death in tumor-bearing mice (4). Scientists decided to change the strain and used non-pathogenic *Clostridium butyricum* M55, its non-pathogenic character speeding the start of clinical studies (5). In 1967 Carey carried out a small experiment with conclusions variating from: without tumor lysis, with tumor lysis and even death (6). Roughly in the same time, for the benefits of amplifying effectiveness of Clostridium, scientist started to combine bacteria with numerous agents, such as heavy metals and classical chemotherapy (7). Many similar researches were carried out later in the 70`s (8; 9).

Dang et al., examined many species targeted at tumors, of which two showed promising effects (10). An ability of targeting the tumor and disseminating in it was found in *Clostridium novyi* and *Clostridium sordellii*. Other than that, these strains were capable of evenly inducing the destruction of surrounding tissue. Despite this, no surprise was the effectiveness of the clostridia led to the death of all animals with tumors. The authors of the experiment had the suspicion, that this toxicity could have been a consequence of toxin secretion. It is commonly known clostridia hold anumber of potentially dangerous genes for toxins. For this reason, *Clostridium novyi* was selected for the purpose of later studies, and was attenuated by elimination of the gene coding the

Author α: Assoc. Prof., DVM, PhD., Department of Biology and Genetics, University of Veterinary Medicine and Pharmacy, Komenskeho 73, Kosice. e-mail: alexandra.valencakova@uvlf.sk
Author σ ρ: Department of Biology and Genetics, University of Veterinary Medicine and Pharmacy, Komenskeho 73, Kosice.
e-mail: elena.hatalova@student.uvlf.sk

lethal NT toxin from its genome. This new strain preserved its capability of targeting the tumor and was still capable of destroying live tumor cells in the proximity of their growth. For the amplification of therapeutic effectiveness, Dang used several chemotherapeutic medications in co-operation with *Clostridium novyi* (10). The association of *C. novyi* with classical chemotherapy brought extreme tumor regression. This type of therapy was named "combination bacteriolytic therapy" (COBALT). Later *in vivo* experiments on a vast scale of tumor cell-lines shown *C. novyi* potentiates the effect of standard radiation modes (11). It was explained lately, that *C. novyi* – NT can be uses as a tool for liposome lysis initiation and can help in liposomal distribution of therapeutic substances to tumors (12). In the clinical study Roberts et al (2014) use volunteers with *C. novyi*–NT.*C. novyi*-NT has been shown in preclinical settings to have excellent tumor colonizing properties (13). Roberts *et al.* use non-armed *C. novyi*-NT bacteria, and it is the specific proteolytic nature of the strain that, once germinated, induces tumor necrosis. Previous studies showed that a single dose of *C. novyi*-NT spores injected intravenously in syngeneic tumor-bearing animals often led to localized tumor necrosis and oncolysis, leading to cures in up to one-third of treated animals, without excessive toxicity (14, 15)].Strains such as *C. sporogenes* also have inherent anti-tumor effects as a consequence of proteolysis, but to a lesser extent, and significant efficacy improvement can be obtained by arming these bugs with additional therapeutic genes. Most studies with armed clostridia have however been performed with so-called prodrug converting enzymes (PCE). Such PCE can convert a non-toxic prodrug into a chemotherapeutic agent (16). Since the PCE is only expressed within the tumor where clostridia reside, the conversion also only takes place locally within the tumor, thereby avoiding the side effects commonly occurring following systemic therapy. In addition, most of these prodrug/PCE combinations are characterized by a potent bystander effect as the converted prodrug can diffuse from the site of conversion towards non-exposed neighbouring cells within its vicinity. The proof-of-principle of this approach has been shown with PCE expressed from a plasmid (17, 18) and more importantly, recently also with a nitroreductase PCE stably integrated into the chromosome (19).

Salmonella: Salmonellae are Gram-negative, facultative anaerobes growing in oxygen-rich conditions as well as oxygen-deficient. When wild type *Salmonella typhimurium* is injected in mice, *Salmonellae* disseminate in the organism and reach high concentrations in the liver (20). Although animals eventually died of organ failure, there was an apparent presence of bacteria in tumors. This observation led scientists to studying the use of *Salmonella* for therapeutic usage against cancer (2).

The modified *Salmonella typhimurium* strain for the uses of cancer therapy was designed at the turn of the century by Vion Pharmaceuticals, Icn. *S. typhimurium* (ATCC 14028) was attenuated in sequence leading to the birth of strain YS1646 (commertial designation VNP20009; 21). This strain was deficient in purine synthesis, which forced the bacteria to use an external source of purines for them to survive. Purine deficiency had two consequences. First, the bacteria became partially attenuated, second, as was observed in mice, proliferation in normal tissues was inhibited, while the capability of proliferation in tumors was preserved. After previous atenuation, the gene coding msbB was removed from the bacterial genome (21). The msbB protein catalyzes the addition of the terminal myristoyl group to lipid A. Lipid A is a component of the lipopolysacharides (LPS) found in Gram-negative bacteria, including *E. coli* and *Salmonella*. During infection, lipid A stimulates the production of cytokines as TNF-α, leading to inflammation and toxic shock. It was proved even earlier, that mutations in the gene coding msbB limited the capability of *Salmonella* to invoke disease, but not its ability to target tumors (22). Toxicity trials after VNP20009 application to mice, rats and small monkeys proved their safe character. This conclusion was verified in the first phase of clinical testing on volunteers (23).

Anti-tumor qualities of strain VNP20009 were also found. It was shown this strain is effective against a vast scale of tumors, as well as against some metastatic lesions (24). But the mechanism of tumor suppression induced by *Salmonella* has still not been explained. One study points to specific genes linked with pathogenicity more than to genes connected with the invasive character of the bacteria (25). However, this theory is in a contrary to evidence of the attenuated *Salmonella* not being directly toxic to tumor cells (26).

Another study shows to the immune system, which can play a key role in tumor suppression. Local inflammatory reactions in subsequence to a large bacterial count in the localization of the tumor were documented. Histological examinations of tumors in mice with B16 melanoma tumor-bearings shown massive neutrophil infiltration as a result of Salmonella application. The bacteria alone can lead to tumor suppression, as was proved in tests on mice with neutrophil depletion (27). More, there is evidence supporting that bacteria can induce toxicity by nitric oxide production specifically in the location of the tumor (28). Besides the mentioned, other bacteria-mediated tumor regression mechanisms were found, for example, toxin secretion and direct competition for nutrition with the tumor cells (2).

Bacteria as gene transport systems

One of the problems connected to the use of bacteria as anti-cancer tools is the toxicity of bacteria in therapeutic dosage. This applies in individual application or in combination with radiation or chemotherapy (10). Reduction of the dosage significantly reduces the toxicity as well as their effect. Some bacteria, such as probiotic bifidobacteria or non-pathogenic bacteria, for example *E.coli* Dh5a can effectively colonize tumors, but they do not have any therapeutic effect due to their non-pathogenic character (2).

The process overcoming both of these limiting factors is to "arm" bacteria with protein coding genes, which can induce cytotoxicity. This provides the therapeutic potential to harmless strains and amplifies effectiveness in more toxic strains. The advantage of this is that in clinical practice a lower and therefore a safer dose of bacteria can be administer do the patient, lowering the systemic toxicity, but maintaining the therapeutic effectiveness in the tumor location (2). A progress in development of *Clostridia* and *Salmonella* strains as non-modified and autonomous anti-cancer pharmaceuticals is expected. In the meantime, many other bacterial strains were developed as tumor interfering agents (29).Some of them are attenuated and some are naturally harmless, as non-pathogenic anaerobic Gram-positive bifidobacteria, belonging to a group of bacteria commonly introduced as lactic acid bacteria or probiotic bacteria, which live in symbiosis in lower parts of the small intestine in humans and other mammals (2).

Bacteria-directed enzyme/prodrug therapy

Bacteria-directed enzyme/prodrug therapy (BDEPT) is found on a process of amplifying effectiveness of bacterial vectors and it reduces therapeutic doses. This procedure uses bacteria for the delivery of the enzyme to the tumor bearings, and involves "arming" bacteria with genes coding an enzyme for transforming the prodrug (that does not have a human homologue and/or has a better enzyme kinetics as a similar human enzyme). BDEPT is a two step therapy. In the first step, the "armed" vector is administered to the patient and it targets specifically in the tumor location, where the enzyme is expressed. In the second step, as soon as the level of enzyme expression is optimal, the predrug is administered and converted by the expressed enzymes to a cytotoxic medicament directly in the tumor location. This leads to a tumor-selective cytotoxicity (2).

There are numbers of homologous therapeutic strategies similar to BDEPT. Antibody-directed enzyme/prodrug therapy (ADEPT) was designed for the first time more than 20 years ago (30, 31). It is based on extracellular targeting of tumor antigens by monoclonal antibodies, chemically connected to a purified predrug-converting enzyme. Many ADEPT systems are being studied; some of them underwent clinical studies (32). Virus-directed enzyme/prodrug therapy (VDEPT) has shown itself as a promising therapeutic method in preclinical and clinical testing (33). Another similar therapy is Polymer-directed enzyme/prodrug therapy (PDEPT; 34), Ligand-directed enzyme/prodrug therapy (35), Melanocyte-directed enzyme/prodrug therapy (MDEPT; 36), and precursor monotherapy (37). The broad term Gene directed enzyme/prodrug therapy (GDEPT) includes all strategies on the principle of gene expression of the precursor-converting enzymes in tumor cells (38). One of the most widely described GDEPT systems became the combination of a *Herpes Simplex Virus*-tymidine-kinase (HSV-tk) nucleoside analog and it dates to the 1980`s (39). The distribution of genes coding HSV-tk*in vivo* was achieved with the use of many vectors, for example: retroviruses, adenoviruses and liposomes (40). In BDEPT method and other precursor-converting methods, the medicament is created *in situ* as a consequence of intervention with the tumor. This grants many advantages with comparison with conventional procedures. High tumor selectivity is achieved, because the precursor is converted only inside the tumor, which reduces side effects in other organs. An amplifying effect is created as a result of the capability of one therapeutic molecule enzyme to activate many prodrug molecules. This leads to high concentrations of active medicament in the location of the tumor. A "bystander effect" is occurring, defined as a capability of bacterial cells to express enzymes stimulating the killing of cells in the proximity of tumor cells not expressing the enzyme. For this reason, bacteria can group to colonies in the stroma of the tumor and they do not need to attack cancer cells for the successful eradication/regression of the tumor (38).

II. Conclusion

In BDEPT the aiming of bacteria to the targeted structures is based on the physical rather than biochemical characteristics of the tumor; non-pathogenic bacteria not toxic for the host can be used; there is a large number of molecular biology techniques using bacteria and they have relatively few obstacles in bacterial gene expression; it is possible to avoid every potential trangene toxicity (which could occur for reasons of striking outside of targeted structures), because genes are enclosed in the bacteria; serum components can`t inhibit enzymes protected by bacterial membranes and cell wall; there is a collection of cofactors as NADH and NADPH which can be used by therapeutic enzymes needing reductive environment; bacteria can be, in difference to viruses, relatively easily reduced in size or modified for clinical uses.

One important difference between BDEPT and other bacterial therapies is, BDEPT uses constitutively toxic genes (for example Salmonella), in BDEPT expressing the apoptotic cytokine Fas ligand the toxicity is controlled and induced after prodrug administration, while in other types of bacterial therapy can be toxic subsequent to injecting to the patient. Systemic toxicity can be induced mostly in the case of bacteria secreting the therapeutic protein. Beside this, bacteria carrying therapeutic genes under the control of eukaryotic promoters can cause problems if the vector targets healthy cells, outcomming as "non-target toxicity". In ideal cases, BDEPT could be combined with imaging technique, so workers in clinical practice could correctly evaluate the aiming to target structures and decide ahead the application of the prodrug.

References Références Referencias

1. Jain RK, Baxter LT. Mechanisms of heterogeneous distribution of monoclonal antibodies and other macromolecules in tumors: significance of elevated interstitial pressure. *Cancer Res.*. (1988) 48: 7022-32.
2. Lehouritis P, Springer C, Tangney M. Bacterial-directed enzyme prodrug therapy. *J Control. Release.* (2013) 170(1): 120-31.
3. Minton NP, Mauchline ML, Lemmon MJ, Brehm JK, Fox M, Michael NP, Giaccia A, Brown JM. Chemotherapeutic tumour targeting using clostridial spores. *FEMS Microbiol. Rev.* (1995) 17:357-64.
4. Malmgren RA, Flanigan CC. Localization of the vegetative form of *Clostridium tetani* in mouse tumors following intravenous spore administration. *Cancer Res.* (1955) 15(7): 473-8.
5. Engelbart K, Gericke D. Oncolysis by clostridia. V. Transplanted tumors of the hamster. *Cancer res.* (1964) 24: 239-42.
6. Carey RW, Holland JF, Sheehe PR, Graham S. Association of cancer of the breast and acute myelocytic leukemia. *Cancer*. (1967) 20(7): 1080-8.
7. Gericke D, Engelbart K. Oncolysis by clostridia. Ii. Experiments on a tumor spectrum with a variety of clostridia in combination with heavy metal. *Cancer Res.* (1964) 24: 217-21.
8. Gericke D, Dietzel F, König W, Rüster I, Schumacher L. Further progress with oncolysis due to apathogenic clostridia. *Zentralbl Bakteriol Orig A*. (1979) 243(1): 102-12.
9. Dietzel F, Gericke D. Intensification of the oncolysis by clostridia by means of radio-frequency hyper-thermy in experiments on animals--dependence on dosage and on intervals (author'stransl). *Strahlentherapie*. (1977) 153(4): 263-6.
10. Dang LH, Bettegowda C, Huso DL, Kinzler KW, Vogelstein B. Combination bacteriolytic therapy for the treatment of experimental tumors. *Proc. Natl. Acad. Sci. USA.* (2001) 98(26): 15155-60.
11. Bettegowda C, Dang LH, Abrams R, Huso DL, Dillehay L, Cheong I, Agrawal N, Borzillary S, McCaffery JM, Watson EL, Lin KS, Bunz F, Baidoo K, Pomper MG, Kinzler KW, Vogelstein B, Zhou S. Overcoming the hypoxic barrier to radiation therapy with anaerobic bacteria. *Proc. Natl. Acad. Sci. USA.* (2003) 100(25): 15083-8.
12. Cheong I, Zhou S. Tumor-specific liposomal drug release mediated by liposomase. *Methods. Enzymol.* (2009) 465: 251-65.
13. Roberts NJ, Zhang L, Janku F, Collins A, Bai RY, Staedtke V, Rusk AW, Tung D, Miller M, Roix J, Khanna KV, Murthy R, Benjamin RS, Helgason T, Szvalb AD, Bird JE, Roy-Chowdhuri S, Zhang HH, Qiao Y, Karim B, McDaniel J, Elpiner A, Sahora A, Lachowicz J, Phillips B, Turner A, Klein MK, Post G, Diaz LA Jr, Riggins GJ, Papadopoulos N, Kinzler KW, Vogelstein B, Bettegowda C, Huso DL, Varterasian M, Saha S, Zhou S. Intratumoral injection of Clostridium novyi-NT spores induces antitumor responses. *Sci. Transl. Med.* (20146: 249ra111.
14. Dang LH, Bettegowda C, Agrawal N, Cheong I, Huso D, Frost P, Loganzo F, Greenberger L, Barkoczy J, Pettit GR, Smith AB 3rd, Gurulingappa H, Khan S, et al. Targeting vascular and avascular compartments of tumors with C. novyi-NT and anti-microtubule agents. *Cancer biology & therapy*. (2004) 3(3): 326-337.
15. Diaz LA, Jr, Cheong I, Foss CA, Zhang X, Peters BA, Agrawal N, Bettegowda C, Karim B, Liu G, Khan K, Huang X, Kohli M, Dang LH, et al. Pharmacologic and toxicologic evaluation of C. novyi-NT spores. *Toxicological sciences: an official journal of the Society of Toxicology.* (2005) 88(2): 562-575.
16. Theys J, Pennington O, Dubois L, et al. Repeated cycles of Clostridium-directed enzyme prodrug therapy resultin sustained antitumour effects in vivo. *Br. J. Cancer* (2006) 95: 1212-9.
17. Liu SC, Ahn GO, Kioi M, et al. Optimized clostridium-directed enzyme prodrug therapy improves the antitumor activity of the novel DNA cross-linking agent PR-104. *Cancer Res*. (2008) 68: 7995-8003.
18. Theys J, Lambin P. *Clostridium* to treat cancer: dream or reality? *Ann. Transl. Med.* (2015) 3(Suppl 1): S21.
19. Heap JT, Theys J, Ehsaan M, et al. Spores of Clostridium engineered for clinical efficacy and safety cause regression and cure of tumors *in vivo*. *Oncotarget* (2014) 5: 1761-9.
20. Bermudes D, Low B, Pawelek J. Tumor-targeted *Salmonella*. Highly selective delivery vectors. *Adv. Exp. Med. Biol.* (2000) 465: 57-63.
21. Low KB, Ittensohn M, Luo X, Zheng LM, King I, Pawelek JM, Bermudes D.Construction of VNP20009: a novel, genetically stable antibiotic-

sensitive strain of tumor-targeting Salmonella for parenteral administration in humans. *Methods Mol. Med.* (2004) 90: 47-60.

22. Low KB, Ittensohn M, Le T, Platt J, Sodi S, Amoss M, Ash O, Carmichael E, Chakraborty A, Fischer J, Lin SL, Luo X, Miller SI, Zheng L, King I, Pawelek JM, Bermudes D. Lipid A mutant *Salmonella* with suppressed virulence and TNFalpha induction retain tumor-targeting *in vivo*. *Nat*. *Biotechnol*. (1999) 17(1): 37-41.
23. Toso JF, Gill VJ, Hwu P, Marincola FM, Restifo NP, Schwartzentruber DJ, Sherry RM, Topalian SL, Yang JC, Stock F, Freezer LJ, Morton KE, Seipp C, Haworth L, Mavroukakis S, White D, MacDonald S, Mao J, Sznol M, Rosenberg SA. Phase I study of the intravenous administration of attenuated *Salmonella typhimurium* to patients with metastatic melanoma. *J. Clin. Oncol.* (2002) 20(1): 142-52.
24. Zheng LM, Luo X, Feng M, Li Z, Le T, Ittensohn M, Trailsmith M, Bermudes D, Lin SL, King IC. Tumor amplified protein expression therapy: *Salmonella* as a tumor-selective protein delivery vector. *Oncol. Res*. (2000) 12(3): 127-35.
25. Pawelek JM, Sodi S, Chakraborty AK, Platt JT, Miller S, Holden DW, Hensel M, Low KB. *Salmonella* pathogenicity island-2 and anticancer activity in mice. *Cancer Gene Ther.* (2002) 9(10): 813-8.
26. Avogadri F[1], Martinoli C, Petrovska L, Chiodoni C, Transidico P, Bronte V, Longhi R, Colombo MP, Dougan G, Rescigno M. Cancer immuno-therapy based on killing of *Salmonella*-infected tumor cells. *Cancer Res.* (2005) 65(9): 3920-7.
27. Westphal K, Leschner S, Jablonska J, Loessner H, Weiss S. Containment of tumor-colonizing bacteria by host neutrophils. *Cancer Res.* (2008) 68(8): 2952-60.
28. Barak Y, Schreiber F, Thorne SH, Contag CH, Debeer D, Matin A. Role of nitric oxide in *Salmonella typhimurium*-mediated cancer cell killing. *BMC Cancer*. (2010) 10:146.
29. Morrissey D, O'Sullivan GC, Tangney M. Tumour targeting with systemically administered bacteria. *Curr. Gene. Ther.* (2010) 10(1): 3-14. Review.
30. Bagshawe KD. Antibody directed enzymes revive anti-cancer prodrugs concept. *Br. J. Cancer.* (1987) 56(5): 531-2.
31. Bagshawe KD. Antibody-directed enzyme/prodrug therapy (ADEPT). *Biochem. Soc. Trans.* (1990) 18 (5): 750-2. Review.
32. Tietze LF, Schmuck K. Prodrugs for targeted tumor therapies: recent developments in ADEPT, GDEPT and PMT. *Curr. Pharm. Des*. (2011) 17(32): 3527-47. Review.
33. Lukashev AN, Lashkevich VA, Ivanova OE, Koroleva GA, Hinkkanen AE, Ilonen J.Recombination in circulating Human enterovirus B: independent evolution of structural and non-structural genome regions. *J. Gen. Virol.* (2005) 86: 3281-90.
34. Satchi-Fainaro R, Hailu H, Davies JW, Summerford C, Duncan R. PDEPT: polymer-directed enzyme prodrug therapy. 2. HPMA copolymer-beta-lactamase and HPMA copolymer-C-Dox as a model combination. *Bioconjug Chem*. (2003) 14(4): 797-804.
35. Spooner RA, Friedlos F, Maycroft K, Stribbling SM, Roussel J, Brueggen J, Stolz B, O'Reilly T, Wood J, Matter A, Marais R, Springer CJ. A novel vascular endothelial growth factor-directed therapy that selectively activates cytotoxic prodrugs. *Br. J. Cancer.* (2003) 88(10): 1622-30.
36. Knaggs S, Malkin H, Osborn HM, Williams NA, Yaqoob P. New prodrugs derived from 6-aminodopamine and 4-aminophenol as candidates for melanocyte-directed enzyme prodrug therapy (MDEPT). *Org. Biomol. Chem*. (2005) 3(21): 4002-10.
37. Tietze LF, Schuster HJ, Schmuck K, Schuberth I, Alves F. Duocarmycin-based prodrugs for cancer prodrug monotherapy. *Bioorg. Med. Chem.* (2008) 16(12): 6312-8.
38. Niculescu-Duvaz I, Spooner R, Marais R, Springer CJ. Gene-directed enzyme prodrug therapy. *Bioconjug. Chem.* (1998) 9(1): 4-22. Review.
39. Moolten FL. Tumor chemosensitivity conferred by inserted herpes thymidine kinase genes: paradigm for a prospective cancer control strategy. *Cancer Res.* (1986) 46(10): 5276-81.
40. Finzi L, Kraemer A, Capron C, Noullet S, Goere D, Penna C, Nordlinger B, Legagneux J, Emile JF, Malafosse R. Improved retroviral suicide gene transfer in colon cancer celll ines after cell synchronization with methotrexate. *J. Exp. Clin. Cancer. Res.* (2011) 30: 92.

This page is intentionally left blank

Global Journal of Medical Research: F
Diseases
Volume 16 Issue 3 Version 1.0 Year 2016
Type: Double Blind Peer Reviewed International Research Journal
Publisher: Global Journals Inc. (USA)
Online ISSN: 2249-4618 & Print ISSN: 0975-5888

Duodenal Brush Border Enzymes in Helicobacter Pylori Infection

By Awad Magbri & Fiona Stevens
Dialysis Access Center of Pittsburgh, United States

Abstract- *Background and Objectives:* H. pylori are an accepted cause of chronic active gastritis and commonly associated with both gastric and duodenal ulcer. Moderate to severe gastritis increases the relative risk of developing peptic ulceration and eradication of the bacteria reduces duodenal ulcer recurrence. The effect of H pylori on the duodenal brush border membrane enzymes have not been studied extensively in this infection. This study evaluates the duodenal brush border enzymes between the H. pylori positive and negatives patients.

Design, setting, participants & measurements: One hundred and nine patients, age range 20-84 years, mean age 56 years were included in the study. They presented to the endoscopy suite of UCHG with upper gastrointestinal symptoms. The duodenal bulb was entirely normal and with no evidence of inflammation on endoscopic examination. Biopsies from the antrum were processed for histology and bacteriological culture. Two biopsies from the duodenal bulb were taken from each patient and were sealed in Para-film and stored at -20C0 until assayed for brush border enzymes.

GJMR-F Classification : *NLMC Code: QW 154*

Duodenal Brush Border Enzymes in Helicobacter Pylori Infection

Awad Magbri [α] & Fiona Stevens [σ]

Abstract- Background and Objectives: H. pylori are an accepted cause of chronic active gastritis and commonly associated with both gastric and duodenal ulcer. Moderate to severe gastritis increases the relative risk of developing peptic ulceration and eradication of the bacteria reduces duodenal ulcer recurrence. The effect of H pylori on the duodenal brush border membrane enzymes have not been studied extensively in this infection. This study evaluates the duodenal brush border enzymes between the H. pylori positive and negatives patients.

Design, setting, participants & measurements: One hundred and nine patients, age range 20-84 years, mean age 56 years were included in the study. They presented to the endoscopy suite of UCHG with upper gastrointestinal symptoms. The duodenal bulb was entirely normal and with no evidence of inflammation on endoscopic examination. Biopsies from the antrum were processed for histology and bacteriological culture. Two biopsies from the duodenal bulb were taken from each patient and were sealed in Para-film and stored at -20C^0 until assayed for brush border enzymes.

Results: Biopsies from duodenal bulb were assayed for the brush border enzymes, alkaline phosphatase (AP), and disaccharides {lactase (lac) and sucrase (Suc)} using the modified method of Dahlqvist and Kelly, micro-plate method as performed by Nugent. They were divided into 2 subgroups, consisting of 60 patients (HP-positive) who had evidence of H. pylori infection on histology and/or on culture and 49 patients (HP-negative) without evidence of H. pylori infection. All patients had normal looking duodenal mucosa on endoscopic examination. The difference in the results of AP activity between the H. pylori positive (mean ± SD 8.26 ± 4.8) and H. pylori negative groups (mean ± SD 9.1 ± 7.7) was highly statistically lower in the former group ($p<0.0001$). The lactase enzyme activity in patients with H. pylori positive (mean ± SD 9.4 ± 8.3) and H. pylori negative (mean ± SD 8.6 ± 7.6) was significant between the groups ($p = 0.036$).

There was no statistically significant difference in the Sucrase activity between H. pylori positive and negative groups (mean ± SD 30.3 ± 22.3 and 28.95 ± 22.1), ($p=0.138$).

Conclusion: AP and lactase enzymes are significantly lower in patients with H. pylori infection and normal duodenal mucosa on endoscopic examination denoting a probable cytopathic effect of the bacterial on the brush membrane enzymes.

Author α: MD, FACP, Dialysis Access Center of Pittsburgh, PA, USA.
e-mail: elmagbri@hotmail.com
Author σ: MD, FRCP, National University of Ireland, Galway.

I. Introduction

Helicobacter pylori are an accepted cause of chronic active gastritis and commonly associated with both gastric and duodenal ulcer. Moderate to severe gastritis increases the relative risk of developing peptic ulceration and eradication of the bacteria reduces duodenal ulcer recurrence (1-4). Hypergastrenemia and increase parietal cell mass or response to stimulation would result in an increase acid load to the duodenum. The resultant gastric metaplasia would be a target of H. pylori infection and the development of duodentitis and duodenal ulcer. The effect of H pylori on the duodenal brush border membrane enzymes have not been studied extensively in patients with normal looking duodenal mucosa. However, brush border enzymes, lactase, sucrase, maltase, leucine amino-peptidase and gamma glutamyl transpeptidase have been studied in the past in various disorders (5-7). This is a prospective longitudinal study of 109 patients presented to University College Hospital Galway, Ireland with upper gastrointestinal symptoms for endoscopy. Duodenal brush border enzymes are measured between the H. pylori positive and negatives to evaluate if there is difference between the 2 groups.

II. Subjects and Methods

One hundred and nine patients, age range 20-84 years, mean age 56 years were included in the study. They presented to the endoscopy suit of UCHG with upper gastrointestinal symptoms. The duodenal bulb was entirely normal and with no evidence of inflammation on endoscopic examination. Biopsies from the antrum were processed for histology and bacteriological culture. Two biopsies from the duodenal bulb were taken from each patient and were sealed in parafilm and stored at -20C^0 until assayed for brush border enzymes.

a) Estimation of duodenal enzymes

The micro-titer plate method for estimation of protein, alkaline phosphatase, and disaccharides (lactase and sucrase) in biopsy materials of human small intestine; a modified method of Dahlqvist et al(8) and Kelly et al(9) as used by D.W. Nugent et al(10), Department of Biochemistry, University College Galway (UCG) was adopted for the assay. The biopsy samples from the duodenal bulb of 109 patients with normal looking duodenal mucosa on endoscopic examination

and with no history of celiac disease or gastro-enteropathies known to affect the duodenal mucosal brush border membrane enzymes. The biopsies were weighted and homogenized in 0.3 ml of distilled water using a Vertis homogenizer (The Vertis Company, Gardiner, New York 12525).

b) Quality controls

Aliquots of pooled biopsy homogenates containing high, medium, and low levels of the enzymes were stored frozen at – 20C^0. An aliquot of each was thawed and included in each batch of samples for analysis.

c) Plate reader

Absorbencies were measured with a Dynatech MR 5000 automatic micro-plate reader (UK) fitted with an appropriate filter. A 410 nm filter was used for alkaline phosphatase assays, 450 nm filter for the disaccharides and 570 nm filter for the protein assays. The results were calculated as described by Nugent et al (10).

d) Protein assays

Homogenate protein estimates were performed using the micro-titer plate method of Nugent et al (10). The reagents necessary for the micro-titer plate method for protein assays are:

- BCA protein assay reagent (code Nr. 23225) pierce contains: Reagent (A) sodium carbonate, sodium bicarbonate, and BCA detection reagent and sodium tartarate in 0.1 M NaOH.
- Reagent (B) is 4% CuSO4.5H2O (10x1 ml ampoules, 2 mg/ml albumin standard).

The methods for protein, alkaline phosphatase, and disaccharides (sucrase and lactase) assays as done by Nugent et al (10) were adapted for calculation of the enzymes.

e) Calculations

The mean sample of absorbance (sample absorbance - blank) is compared to the standard curve.

Enzyme Unit/ml sample = μmoles p-nitrophenol/ml
Enzyme Units/gram protein = Enzyme unit/ml sample ÷ protein concentration of sample (mg/ml).

f) Disaccharides assay calculations

The unit of lactase and sucrase activity is defined as the amount of the enzyme that will liberate one μmole of glucose from the μmole of substrate per minute under the assay conditions specified.

Lactase and sucrase activities are expressed as units/g homogenate protein.

The mean sample absorbance (sample absorbance – sample blank) is compared to the standard curve.

Enzyme units/ml sample = μmole glucose/ml ÷ 60
Enzyme units/g protein = Enzyme units/ml sample ÷ protein concentration of sample (mg/ml).

g) Statistical methods

The results of brush border enzyme analysis were analyzed using the Mann-Whitney U test. We used the SYSTAT computer package (SYSTAT 1990, inc). The difference in the alkaline phosphatase, sucrase and lactase between H. pylori positive and negative was estimated. A p value of 0.05 was taken as significant.

III. Results

Biopsies from duodenal bulb of 109 patients were assayed for the brush border enzymes using the modified method of Dahlqvist et al (8) and Kelly et al (9), micro-plate method as performed by Nugent et al (10). They were divided into 2 subgroups, consisting of 60 patients (HP-positive) who had evidence of H. pylori infection on histology and/or on culture and 49 patients (HP-negative) without evidence of H. pylori infection. All patients had normal looking duodenal mucosa on endoscopic examination. The difference in the results of alkaline phosphatase activity between the H. pylori positive (mean ± SD 8.26 ± 4.8) and H. pylori negative groups (mean ± SD 9.1 ± 7.7) was highly statistically lower in the former group ($p<0.0001$).

The lactase enzyme activity in patients with H. pylori positive (mean ± SD 9.4 ± 8.3) and H. pylori negative (mean ± SD 8.6 ± 7.6) was significant between the groups ($p = 0.036$).

There was no statistically significant difference in the sucrase activity between H. pylori positive and negative groups (mean ± SD 30.3 ± 22.3 and 28.95 ± 22.1), ($p=0.138$).

IV. Discussion

H. pylori is capable of inflecting a cytopathic effects on the gastro-duodenal mucosa either through the release of substances secreted by the bacteria like phospholipase A and its cytotoxic metabolites, lysolecithin, which was found to be high in patients with H. pylori infection (11) and/or through the stimulation of the body immune response, both cellular and humoral components.

In this study the effect of antral H. pylori colonization on duodenal brush border enzymes has been investigated. The level of alkaline phosphatase and lactase were significantly lower in patients with H. pylori infection. There was no significant difference in the level of sucrase enzyme between the 2 groups. There are scarce reports of similar studies on endoscopically normal duodenal mucosa for comparison. The first data on the duodenal mucosal enzyme activities in patients with duodenal ulcer was reported by Vetvik et al (12). These investigators have found that most membrane enzymes activities were decreased in the duodenum of DU patients. Their findings are not unexpected due to the cytopathic effects on the cell membrane of the lysosomal enzymes and other inflammatory mediators

from the local inflammatory infiltrate. This process may disrupt the attachment of the membrane brush border enzymes. Our results were the first to demonstrate decreased membrane enzyme activities in patients with H. pylori infection and with endoscopically normal mucosa.

The results of alkaline phosphatase and lactase in this study suggest that the organism H. pylori may have cytopathic effect on the brush border cells of the duodenal mucosa through the release of substances like phospholipase A2 and its toxic metabolite, lysolecithin. These substances are reported by Langston et al (11) to be higher in patients with H. pylori infection. Recent quantitative histological analysis of duodenal biopsies of dyspeptic patients with no endoscopic duodenitis has shown increased polymorph and mononuclear cell infiltrate in duodenal bulb mucosa relative to controls (13, 14) suggesting subclinical inflammation which is insufficient to produce a lesion recognizable endoscopically (13). Toxic substances may be carried from the antrum to exert their effect on the duodenal mucosa even before the macroscopic appearance of duodenal inflammation. The nature of these substances needs to be further elucidated and characterized. The disruption of the cyto-skeletal membranes of the duodenal cells results in loss of membrane-bound duodenal enzymes. Consequently, this may have other deleterious effect on the integrity of the duodenal cells either directly or indirectly through the chemical substances like bile acids and the alkaline nature of the environment as a result of elevated pH. The inflammatory process which develops through the activation of the local and systemic components of the immune system of the infected individual will further contribute to the duodenal mucosal damage. The inflammatory process is probably important for H. pylori colonization of the duodenal mucosa. Healing of these inflammatory areas result in the development of gastric metaplasia in the duodenum. The gastric metaplasia may then be colonized by H. pylori. The limitations of this study are that it was carried out on a small sample size and in one center which may affect its significance. Repeating the study on a large sample size would add weight to the validity of the results.

V. Conclusion

Alkaline phosphatase and lactase enzymes are significantly lower in patients with H. pylori infection and normal duodenal mucosa on endoscopic examination denoting a probable cytopathic effect of the bacterial on the brush membrane enzymes.

References Références Referencias

1. Bell GD & Powell KU. Eradication of Helicobacter pylori and its effect in peptic ulcer disease. Scan J Gastroenterol. 1993; (suppl 196): 7-11.
2. Bell GD, Powell KU, Burridge SM, et al. Reinfection or recurrence after successful eradication of Helicobacter pylori infection: implication for treatment of patients with duodenal ulcer disease. Quart J Med. 1993; 86: 375-82.
3. Bayerdorffer E, Mannes GA, Sommer A, et al. Long-term follow up after eradication of Helicobacter pylori with a combination of omeprazole and amoxicillin. Scand J Gastroenterol. 1993; (suppl 28): 196: 19-25.
4. O'Morain C, & Gilvarry J. Eradication of Helicobacter pylori in patients with non-ulcer dyspepsia. Scand J Gastroenterol. 1993; (28 suppl) 196: 30-33.
5. Katyal R, Rana SV, Vaiphei K, et al. Effect of rotavirus infection on small gut pathophysiology in a mouse model. J Gastroenterol Hepatol. 1999; 14: 779-794.
6. Rana SV, Gupta D, Malik R, et al. Mild to moderate malnutrition and small intestine of young rhesus monkeys. Nutrition. 1995; 11: 292-295
7. Fernandes VLC, Bhasin DK, Rana SV. Study of enzyme activities in the descending part of the duodenum in patients of duodenal ulcer. Indian J Clin Biochemistry. 2006; 21(1): 169-172.
8. Dahlqvist A. Assay of intestinal disaccharides. Analyt Biochem. 1968; 22: 99-107.
9. Kelly MH, Hamilton JR. A micro-technique for the assay of intestinal alkaline phosphatase. Results in normal children and children with celiac disease. Clin Biochem. 1970; 3: 33-43.
10. Nugent DW, Doyle C, Fottrell PF. Micro-titer plate method for estimating protein in biopsies of human small intestine. Clin Chemistry. 1987; 33(9): 1671
11. Langston RS, Cesareo SD. Helicobacter pylori associated phospholipase A2 activity: a factor in peptic ulcer production? J Clin Pathol 1992; 45(3): 221-224.
12. Vetvik K, Schrumpf E, Andersen KJ, Borkje B, et al. Enzyme activities in the duodenal mucosa in duodenal ulcer patients. Scand J Gastroenterol. 1989; 24: 244-50.
13. Collins JSA, Hamilton PW, Watt PCH, et al. Quantitative histological study of mucosal inflammatory cell densities in endoscopic duodenal biopsy specimens from dyspeptic patients using computer linked image analysis. Gut. 1990; 31: 858-861.
14. Mollenkopf C, Steininger H, Weineck G., et al. Gastritis: immunohistochemical detection of specific and nonspecific immune response to Helicobacter pylori. Zeitschrift Fur Gastroenterol. 1990; 28(7): 327-334.

This page is intentionally left blank

Global Journal of Medical Research: F
Diseases
Volume 16 Issue 3 Version 1.0 Year 2016
Type: Double Blind Peer Reviewed International Research Journal
Publisher: Global Journals Inc. (USA)
Online ISSN: 2249-4618 & Print ISSN: 0975-5888

Comparison of Binary Models for the Associated Factors Affecting Recovery Status of Vesicovaginal Obstetrics Fistula Patients: A Case of Mettu Hamlin Fistula Center, South West Ethiopian

By Aboma Temesgen
Haramaya University

Abstract- *Back ground:* Obstetric fistula or vaginal fistula is a medical condition in which a fistula (hole) develops between either the rectum and vagina or between the bladder and vagina after severe or failed childbirth, when adequate medical care is not available. It is the most tragic of preventable childbirth complications in the developing world, as affected women are often abandoned by their husbands and family, and forced to live in shame.

Objective: The main objective the study was to determine an appropriate binary model for the recovery status of the vesicovaginal patients. Further more, the study explores factors affecting the recovery status of the patients during the time period of the study.

Methods: The study consists of 206 vesicovaginal fistula patients having all required information who were taking treatment at Metu Hamlin Fistula center from November 2010 to June 2014. The chi-square test of association were employed to explore the association between the recovery status and categorical independent variables.

Keywords: *binary data modeling, akaki information criteria, link function, vesicovaginal fistula.*

GJMR-F Classification : *NLMC Code: WJ 190*

Comparison of Binary Models for the Associated Factors Affecting Recovery Status of Vesicovaginal Obstetrics Fistula Patients: A Case of Mettu Hamlin Fistula Center, South West Ethiopian

Aboma Temesgen

Abstract- Back ground: Obstetric fistula or vaginal fistula is a medical condition in which a fistula (hole) develops between either the rectum and vagina or between the bladder and vagina after severe or failed childbirth, when adequate medical care is not available. It is the most tragic of preventable childbirth complications in the developing world, as affected women are often abandoned by their husbands and family, and forced to
live in shame.

Objective: The main objective the study was to determine an appropriate binary model for the recovery status of the vesicovaginal patients. Further more, the study explores factors affecting the recovery status of the patients during the time period of the study.

Methods: The study consists of 206 vesicovaginal fistula patients having all required information who were taking treatment at Metu Hamlin Fistula center from November 2010 to June 2014. The chi-square test of association were employed to explore the association between the recovery status and categorical independent variables. After exploring the association between the variables, different binary models were employed to have an appropriate model for the recovery status of the patients based on Akaki information criteria of the model.

Results: The chi-square test of association showed that width of fistula, length of fistula and bladder size categories were significantly associated with recovery status of the patients at 5% of level of significance. The study showed among the candidate binary models logistic model was considers an appropriate model. Furthermore, the fitted model showed width, length of fistula and bladder size categories were the factors that have significant effect on the recovery status of the patients at 5% level of significance.

Conclusion: Logistic regression model was the better fit of the data and the fistula patients with width and length fistula category group between three up to five centimeter were less likely to be recovered recomparison with the fistula patients group with width and length of fistula less than or equal to two centimeters. Similarly, the none bladder size category patients where less likely recover in comparison with fair bladder size fair bladder size group patients.

Author: Department of Statistics, Haramaya University, Dire Dawa.
e-mail: aboma.temesgen@gmail.com

Keywords: binary data modeling, akaki information criteria, link function, vesicovaginal fistula.

I. Introduction

Obstetric fistula or vaginal fistula is a medical condition in which a fistula (hole) develops between either the rectum and vagina or between the bladder and vagina after severe or failed childbirth, when adequate medical care is not available. It is considered a disease of poverty because of its tendency to occur in women in poor countries who do not have health resources comparable to developed nations[1].

It is classically regarded as an accident of childbirth in which prolonged obstructed labor leads to destruction of the vesicovaginal/rectovaginal septum with consequent loss of urinary and/or fecal control. Obstetric fistula is highly stigmatizing and afflicted women often become social outcasts. Although obstetric fistula has been eliminated from advanced industrialized nations, it remains a major public health problem in the worlds poorest countries. Several million cases of obstetric fistula are currently thought to exist in sub-Saharan Africa and south Asia[2].

Immediate causes for vesicovaginal fistula may be obstructed labor, pelvic surgery, and sexual abuse before reaching physical maturity, malignancy, radiotherapy or a combination of these. In most third world countries over 90% of fistulas are of obstetric nature and usually caused by obstructed labor[3]. Whereas Primary risk factors include early or closely spaced pregnancies and lack of access to emergency obstetric care Women affected with Crohn's disease also have a higher risk of developing obstetric fistulas. It mainly occurring amongst the illiterate farmers after prolonged obstructed labor. Public enlightenment and appropriate ante-natal care and delivery would reduce the incidence[4].

Obstetric fistula is the most tragic of preventable childbirth complications in the developing world, as affected women are often abandoned by their

husbands and family, and forced to live in shame. They occur almost entirely in the developing world and their incidence is poorly studied. As obstetric fistula is a serious preventable public health issue in developing countries, national and international organizations should launch a campaign to end fistula by increasing the resources and skilled staff available locally to treat obstetric fistula for improving the lives of women currently living with this condition. Moreover, effective preventive strategies for obstetric fistula such as better education to women and provision of improved obstetric care and searching for the best approaches to both prevention and treatment should be the priority[5].

Besides physical treatment, mental health services are also needed to rehabilitate fistula patients, who experience a great deal of psychological trauma from being ostracized by her community and from fear of developing fistula again. A study on the first formal counseling program for fistula survivors in Eritrea shows positive results, whereby counseling significantly improved the women's self-esteem, knowledge about fistula and fistula prevention, and behavioral intentions for health maintenance and social reintegration following surgery[6]. Cross-sectional study in Ethiopia also showed fistula prevalence of the disease among19,153 households with 97,765 inhabitants surveyed fifty-five women with fistula were identified, of which 39 untreated. The overall estimated prevalence of fistula was 2.2 per 1000 women[7].

a) Binary data modeling

The binary model is among generalized linear models in which the outcome variables are measured on a binary scale that is the responses may be form of recovered or not recovered, alive or dead and present or absent. This binary data modeling is widely used in medical literature especially for correlating the dichotomous outcomes with the predictor variables that include different physiological data. In case of binary logistic regression, the predicted odd ratio of positive outcome is expressed as a sum of product. Product is formed by multiplying the values of independent variable and its coefficients. The probability of positive outcome is obtained from the odd ratio through a simple transformation [8,9].

A logistic regression model is the result of non-linear transformation of the linear regression model. The difference between logistic regression and linear regression is that the outcome variable in logistic regression is dichotomous [10]. Many medical research problems call for the analysis and prediction of a dichotomous outcome: whether smokers will have a chance of developing lung cancer, hyperuricemia patients have the risk of getting cardio vascular disease. Traditionally, these research questions were addressed by either ordinary least squares (OLS) regression or linear discriminant function analysis[11].

The recovery status of the patient from the vesicovaginal obstetrics fistula was also one of the binary outcome variables that is the patients recovered or not recovered from the disease after getting treatment during the study period. How ever, the previous studies conducted on vesicovaginal fistula was on the measures of prevalence the diseases in Ethiopian context rather than modeling to determine factors related to the prevalence of fistula. Therefore, the main aim of this study was to investigated an appropriate binary model for the recovery status of the vesicovaginal patients in the study area. Further-more, the study also explores factors that determine the recovery status of the patients.

II. Methodology

a) Data sources

Data for this study was obtained from the retrospective institutional based study conducted on mothers came for fistula repair at Mettu Hamlin fistula center from November 2010 to June 2014 G.C. The study considered all women who visited at the center having vesicovaginal fistula case only during the time period as study population. Among the total of 585 women patients visited from the year November 2010 to June 2014 G.C 206 having complete information were considered in the study.

i. *Variables of the study*

The outcome variable of the study was the recover status from vesicovaginal fistula patients which indicates wether the patient was recovered from the vesicovaginal fistula or not recovered during the time period. A patient is said to be recovered if she physically cured from her sickness and no requirement for intervention of health care professionals otherwise we call it the patient is not recovered during the study period.

The study also considered 19 independent variables to know their significant effects on the recover status of the patients. The list of this independent variables with respective of their categories was given on the table one below.

Table 1 : List of independent variables considered for the study

Variable	Categories of variables with their codes
Age at first marriage	0 =≥15, 1 =16-19, 2 =≥20 years
Age at occurrence of VVF	0 = ≤20, 1 =21-29, 2 =≥ 30 years
Weight of patient at arrival in MHFC	0 =< 50,1 = ≥50kg
Parity	0 =primipara,1 =multipara,2 =Grandmultipara,3 =Nuli para
Educational status	0 = illiterate, 1 =literate
Marital status	0 =single,1 =married,2 =divorced,3 =widowed, 4 =separated
Accompanying person	0 =self,1 =husband,2 =relatives,3 =husband and relatives, 4 =others
Antenatal care	0 =yes, 1 =no
Place of delivery	0 =home, 1 =health institution,2 =other
Mode of delivery	0=vaginal, 1=others
Duration of incontinence	0 = ≤3, 1 =4-6, 2 =≥7 month
Duration of labor	0 =≤ 2, 1 =3,2 = ≥ 4 day
Fetal outcome	0 =still birth, 1 =alive,2 = early neonatal, 3 = dead
Width of fistula hole	0 = ≤2, 1 =3-5, 2 => 5cm
Length of fistula hole	0 = ≤2,1 = 3-5,2 = >5cm
Status of urethra	0 =intact, 1 =partially damaged, 2 =complete destructed)
Bladder size	0 =none, 1 =small, 2 =fair, 3 =good, 4 =no information)

b) Binary data analysis

It is common to have binary outcome variables in many medical cases that is a patient get cured or not, recovered or not and died or not from their disease after getting certain treatments. This study also deals with recovery status of the vesicovaginal patient after getting treatment to know whether the patient is recovered or not during the study period. That is the outcome variable considered for the study was binary in nature as stated under the variables of the study section of the methodology part. Therefore, the outcome variable takes the value of 1 if the patients recovered from vesicovaginal fistula after getting treatment during the time period and 0 if the patient is note recovered from the vesicovaginal fistula obstetrics after having treatments during specified time period.

As the principal objective of this study was to investigate an appropriate binary model data in order to determine the relationship between the outcome variable with different predictor variables. This help us to know the effect of this predictor on probability of recovering from vesicovaginal fistula. That is the out come variable have binomial distribution with recovery from fistula obstetrics as success and not recovering from specified disease as failure event which can be expresses as follows:

$$Y_i = \begin{cases} 1, & \text{for recovered patients} \\ 0, & \text{other wise} \end{cases}$$

Where; Yi is the treatment outcome for the i^{th} patient

i. *Chi-square test of association*

Before proceeding to the binary data modeling the study explored the association between the outcome variable and different categorical covariates using chi-square test of association. This test statistic was considered for the study to verify whether the categorical covariates and the outcome variable are independent or not.

Hypothesis to be tested for the study was expressed as: Null hypothesis which states as the recovery status of the patients and the independent categorical variables are independent versus the alternative hypothesis which states the recovery status and categorical independent variables are dependent (not the null hypothesis) where the test statistics to test the hypothesis was expresses as:

$$\chi^2 = \sum_{j=1}^{r}\sum_{i=1}^{c} \frac{(0_{ij} - E_{ij})^2}{E_{ij}} \sim \chi_{(r-1)(c-1)}(\alpha) \quad (1)$$

Where; Oij is observed value in jthrow and ithcolumn where as $E_{ij} = \frac{rowtotal * columntotal}{grandtotal}$

which have chi-square distribution. Moreover, it can be verified as there is an association if the computed chi-square value is greater than the critical value of chi-square or if the computed probability of observing greater computed chi-square value (p-value) is less than the level of significance(alpha value). Meaning that the categorical outcome variable and the categorical independent variables were dependent.

ii. *Modeling the recovering status from vesicovaginal fistula*

After identifying the associations between the predictors (independent variables) and the outcome variable using the chi-square test of independency the next step is to model the recovery status of the patients. This fitted model helps us to know the effects of the predictors which have an association on the probability

of recovering from the diseases. Therefore, to have an appropriate model that represents recovery probability of the patients the study considered probit, complementary log-log and logistic regression models. Whereas the probability of recovering from the diseases was expressed as follows in case of logistic regression with probit link function:

$$\pi_i = \frac{exp(\mathbf{X}^T\beta)}{1+exp(\mathbf{X}^T\beta)} \qquad (2)$$
$$\eta = Log(\frac{\pi i}{1-\pi_i}) = \mathbf{X}^T\beta$$

Where; η is the logit link functions that associate the outcome variables with predictor variables as linear relation, X is nxp matrices of predictors and β is px1 vectors of coefficients for the predictor variables. Furthermore, there are also alternative way of modeling probability of recovering from the diseases in case of using probit and logit link functions where they can be expressed as:

$$\pi_i = \phi = \int_{-\infty}^{\mathbf{X}^T\beta} \frac{1}{\sqrt{2\pi}} exp(-\frac{1}{2}z^2)dz \qquad (3)$$
$$\eta = \phi^{-1}(\pi_i) = \mathbf{X}^T\beta$$

Where; here η is the probit link functions that associate the outcome variables with predictor variables as linear relation using the inverse of cumulative standardized normal distribution whereas the complementary log log link function is expressed as follows:

$$\pi_i = 1 - exp(-exp(\mathbf{X}^T\beta) \qquad (4)$$
$$\eta = log(-log(1-\pi_i) = \mathbf{X}^T\beta$$

iii. *Model estimation techniques*

The outcome variable which represents the treatment outcome of the patients are assumed to be the observed values of independent Bernoulli random variables $y_1, y_2, ..., y_n$ such that y_i has the binomial distribution since it is observed from the n^{th} Bernoulli process randomly. When these binary data are grouped by covariate class, the outcome variable have the form $\frac{y_i}{m_i}, ..., \frac{y_n}{mn}$, where $0 < yi < m_i$, is the number of successes out of the m, subjects in the ith covariate class. The vector of covariate class sizes m $= (mi, ..., m_n)$ is called the binomial index vector or binomial denominator vector. Therefore, Y_i indexed by mi with parameter π_i probability of recovering from VVF where its log likelihood functions is expressed as:

$$L(y_i, \pi_i) = \prod_{i=1}^{n} P(y_i) = \prod_{i=1}^{n} \binom{m_i}{y_i} \pi^{y_i}(1-\pi_i)^{m_i - y_i} = \prod_{i=1}^{n} \binom{m_i}{i} (\frac{\pi_i}{1-\pi_i})^{y_i}(1-\pi_i)^{m_i} \qquad (5)$$

Where the log likelihood function has the following forms:

$$l(y_i, \pi_i) = \sum_{i=1}^{n} (\binom{m_i}{y_i} + y_i log(\frac{\pi_i}{1-\pi_i}) + m_i log(1-\pi_i)) \qquad (6)$$

The constant function of y_i not involving π_i, that is $\binom{m_i}{y_i}$ has no role in the estimation of π_i we remove from the log likelihood function is reduced to:

$$l(y_i, \pi_i) = \sum_{i=1}^{n} (y_i log(\frac{\pi_i}{1-\pi_i}) + m_i log(1-\pi_i)) \qquad (7)$$

But here we consider the three systematic parts (link function) of the model specifies the relation between the π_i and the matrices nxp of covariates X. consider the case of logil link function where; $\eta = log(\frac{\pi_i}{1-\pi_i})$ in which $\pi_i = \frac{exp(X^T\beta}{1+exp(X^T\beta}$

$$l(\beta, y_j) = \sum_{i=1}^{n}\sum_{j=1}^{r} y_i X_{ij}\beta_j - \sum_{i=1}^{n} m_i(1+exp(\sum_{j=1}^{r} X_{ij}\beta_j)) \qquad (8)$$

which obtained by substituting the value of probability of recovery from the logit link function where β_j rx1 vectors of the coefficient for the covariates which is obtained by maximizing the log likely hood function with respect to β_j values. Similarly the loglikely hood function for the probit and complement lo-log link function is obtained by substituting the value of the probability of recovery from the given link function to estimate the parameters of the model.

iv. *Model adequacy tests*

To have appropriate predictors as well as appropriate link function for the recovery probability from Visco Vaginal fistula various model selection criteria was considered which was discussed here under the following

The Wald test: TheWald test was considered to test the significance of individual parameter in the estimated

model to identify whether the covariate have significant effect on the recovery visco vaginal fistula or not.

The fore the wald test statistics was expresses as:

$$Z = \frac{\beta_j}{SE(\beta_j)} \backsim N(0,1) \quad (9)$$

Alternatively, we can treat the square of this statistic as approximately a chi-squared with one degree of freedom which has the following form that is $Z^2 \backsim \chi_1^2$

Depending on the wald test statistics if the covariates do not have significant on the recovery status of the patients that covariate was removed from the model depending on the values of computed Wald test statistics.

Deviance: deviance value is another selection criteria considered for the model selection where it values is based on log likelihood and the model with minimum values of deviance statistics was considered as an appropriate model. The deviance value is used to compare nested models where the deviance value of a model expressed as:

$$D = 2\phi(l_{full} - l_{current}) \quad (10)$$

Where, ϕ represent the scale parameter $l_{current}$ the log-likelihood of the current fitted model and lfull is the log-likelihood of the saturated model.

The Akaike information criterion (AIC): Akaike information is also another model selection criteria considered for the selection of appropriate model as well as appropriate predictor covariates for probability of recover from VVF in the study area. Where the values of AIC is expressed as:

$$AIC = -2log(likelyhood) + 2p \quad (11)$$

Where, log(likelihood) is the log likelihood function which measures the goodness of the fitted model where as p is the number of estimated parameter in the model which measures the how complexity of fitted model. We model with minimum values of AIC was considered as an appropriate model.

III. Results

a) Descriptive result

Among total of 206 vesicovaginal fistula patients considered from November 2010 to June 2014 at Mettu Hamlin fistula Hospital most of the patients (157 (76.21%)) of the patients were recovered whereas only 49(23.79%) were not recovered during the follow up period. The age at occurrence of fistula category with the recovery status of patients also shows most of (63(40.1%)) were the patients infected with the disease at an age of less than or equal to 15 years whereas most none recovered patients (21(42.9%)) also belongs to this age category group.

The age at marriage description also shows most of the patients (26(53.1%)) of none recovered and most (72(45.9%)) recovered patients married at an age of less than or equal to 15 years. Similarily, 11(22.4%) of none recovered and 35(22.3%) of the recovered patients married at an age of greater than or equal to 20 years and between 16 and 19 years respectively which represents smaller proportion in comparison with other marriage age category groups.

Among the total patients considered in the study 30(61.2%) among the none recovered patients and 93(59.2%) among the recovered patients gets antenatal care which represents larger proportion of the patients in both groups in comparison with patients who had no antenatal care. Duration of lasting inconsistence description also indicates 29(59.2%) among the none recovered patients and 88(56.1%) recovered patients lasted duration of inconsistency between four up to six months in comparison with remaining periods of inconsistency groups. Similarily, 30(61.2%) among the none recovered fistula patients and 8(51.6%) among the recovered patients lasted duration of labor up to greater than four days which represents larger proportion in comparison with other categories of duration of labor.

Similarly the place of delivery of patients shows for both recovered and none recovered patients most 26(53.1%) of the none recovered patients and most 104(66.2%) among the recovered patients give the delivery at their home which represents the larger proportion of the patients in both cases in comparison with patient group who gave delivery at health institution.

It can be also observed from the table 2 that among none recovered patients most 32(65.3%) of and among the recovered patients 138(87.9%) of them have length of fistula less than or equal to two centimeter which is larger proportion in comparison with remaining fistula length patients groups. Similarly as observed from the urethra status of the patients more than half of the urethra of none recovered patients were partially damaged whereas larger 97(61.8%) proportion of recovered patients urethra was intact in comparison with the remaining status of urethra groups.

Table 2 : Description of independent variables with recovery status of the patients

Covariates	Categories	Recovery status		Total n(%)
		Not recovered n(%)	Recovered n(%)	
Age at occurrence of fistula	<= 15 years	21(42.9)	63(40.1)	84(40.78)
	16-19 years	18(36.7)	56(35.7)	74(35.92)
	>= 20 years	10(20.4)	38(24.2)	48(23.30)
Age at marriage	<= 15 years	26(53.1)	72(45.9)	98(47.57)
	16-19 years	12(24.5)	35(22.3)	47(22.82)
	>= 20 years	11(22.4)	50(31.8)	61(29.61)
Height	<= 150 Centimeter	44(89.8)	129(82.2)	173(83.98)
	> 150 Centimeter	5(10.2)	28(17.8)	32(15.53)
Weight	< 50 kilogram	43(87.8)	117(74.5)	160(77.67)
	>=50 kilogram	6(12.2)	40(25.5)	46(22.33)
parity	Primipara	4(8.2)	21(13.4)	25(12.14)
	Multipara	28(57.1)	76(48.4)	104(50.49)
	Grandmultipara	17(34.7)	60(38.2)	77(37.38)
Educational status	Illiterate	10(20.4)	50(31.8)	60(29.13)
	Literate	39(79.6)	107(68.2)	146(70.87)
Marital status	Single	4(8.2)	6(3.8)	10(4.85)
	Married	28(57.1)	104(66.2)	132(64.08)
	Divorced	11(22.4)	39(24.8)	50(24.27)
	Separated	6(12.2)	8(5.1)	14(6.80)
Accompanying person	Self	2(4.1)	6(3.8)	8(3.88)
	Husband	15(30.6)	47(29.9)	62(30.10)
	Relative	10(20.4)	39(24.8)	49(23.79)
	Husband and relatives	4(8.2)	6(3.8)	10(4.85)
	Others	18(36.7)	59(37.6)	77(37.38)
Antenatal care	Yes	30(61.2)	93(59.2)	123(59.71)
	No	19(38.8)	64(40.8)	83(40.29)
Duration of incontinence	<= 3month	8(16.3)	24(15.3)	32(15.53)
	4-6 month	29(59.2)	88(56.1)	117(56.80)
	>= 7 month	12(24.5)	45(28.7)	57(27.67)
Duration of labor	<= 2 days	14(28.6)	52(33.1)	66(32.04)
	3-4 days	5(10.2)	24(15.3)	29(14.08)
	>= 4 days	30(61.2)	8(51.6)	38(18.45)
Place of delivery	Home	26(53.1)	104(66.2)	130(63.11)
	Health institutions	23(46.9)	53(33.8)	76(36.89)
Mode of delivery	Vaginal	16(32.7)	68(43.3)	84(40.78)
	Others	33(67.3)	89(56.7)	122(59.22)
Fetal out come	Still birth	43(87.8)	139(88.5)	182(88.35)
	Alive	6(12.2)	18(11.5)	24(11.65)
Length of fistula hole	<= 2centimeter	32(65.3)	138(87.9)	170(82.52)
	3-5 centimeter	17(34.7)	19 (12.1)	36(17.48)
Width of fistula	<= 2 centimeter	25(51.0)	118(75.2)	143(69.42)
	3-5 centimeter	24(49.0)	39(24.8)	63(30.58)
Bladder size	None	25(51.0)	101(64.3)	126(61.17)
	Small	18(36.7)	52(33.1)	70(33.98)
	Fair	6(12.2)	4(2.5)	10(4.85)
Status of urethra	Intact	24(49.0)	97(61.8)	121(58.74)
	Partially damaged	25(51.0)	60(38.2)	85(41.26)
Total	——	49(23.79)	157(76.21)	206(100.00)

b) Exploring association between the recovery status and independent variables

Before proceeding to the binary modeling first of all chi-square test of association were employed to explore whether the independent covariates have an association with treatment outcome or not. Therefore, the chi-square test result of table 3 showed that among all covariate considered in the study length of fistula, bladder size and width fistula categories have significant association with the recovery status of the patients at 5% level of significance since the probability of getting larger computed chi-square value for these covariates were smaller than 5% level of significance. Similarily, weight categories and place of delivery were significance associated with recovery status at 10% level of significance since of the probability of observing larger computed value of chi-square for these covariates were lesser than 10% level of significance.

Table 3 : Chi-square test results for independence

Covariate	DF	Chi-square value	p-value	Covariate	DF	Chi-square value	p-value
Current age	2	0.311	0.856	Age at marriage	2	1.600	0.499
Height	1	1.616	0.204	Weight	1	3.77	0.052*
Parity	2	1.524	0.467	Education	1	2.367	0.124
Marital status	3	4.829	0.185	Accompany person	4	1.778	0.777
Antenatal care	1	0.061	0.804	Duration of inconsistency	2	0.326	0.804
Duration of labor	2	1.57	0.456	Place of delivery	1	2.787	0.095*
Mode of delivery	1	1.757	0.185	Fetal outcome	1	0.022	0.882
Length of fistula	1	13.217	0.000**	Width of fistula	1	10.250	0.001**
Bladder size	2	8.459	0.015**	Status of urethra	1	2.526	0.112

NB:** Indicates significance at 5% level of significance,* Indicates significance at 10% level of significance

c) Comparison of binary models

After exploring the association between the recovery status and independent variables the three commonly used link function were considered for the binomial distribution model. Therefore; all the three model were fitted with length of fistula, bladder size, weight of the patients, place of delivery and width of fistula as independent covariate since they have significant association with outcome variable the result of the model was also follows.

As it can be observed from the log likelihood function and AIC of the three fitted models there is no this much bigger model criteria difference between the three fitted model results. But when we compare them the model with logit link function have smaller negative values of log likelihood and AIC value than probit and complementary log-log link functions. Therefore, logit link function was considered as an appropriate link function to fit data in the study area than the probit and complementary log-log link functions.

Table 4 : Comparison of the models

Link function	DF	Log likelihood function	AIC
Logit	6	-100.771	213.541
Probit	6	-101.029	214.058
Clog-log	6	-101.140	214.280

d) Factors affecting the recovery status of the patients

The logistic model was fitted with an appropriate covariates and weight of the patients were removed from the model because of it do not have significant effects on the model. This model was fitted with width of fistula, length of fistula, bladder size and place of delivery as independent covariates. Among all the covariate considered in the model only place of delivery categories do not have significant effects on the recovery status at 5% level of significance.

The estimated coefficient of logit model for the width of fistula category group three up to five centimeter -0.774 indicates that log of odd ratio of recovery was 0.774 lower in this category group in comparison with patient group with fistula width less than or equal to two centimeter. Similarly, the estimated logit coefficient of bladder size with small category group -0.245 shows the log f odd ratio of patient group with bladder size small was 0.245 lower where as estimated coefficient -1.622 for fair bladder size shows the log of odd ratio of patient group in this category group have 1.622 lower in comparison with none bladder size patient category.

The estimated odd ratio for three up to five width fistula 0.461 shows the odds recovery in this category group was 0.461 times less likely than the patient group with width of fistula less than or equal to two centimeter. Similarly, the estimated odd ratio for fair bladder size patients 0.197 also show that the odds of recovery in this category group group was 0.197 time that of none bladder size patient groups that is the fair bladder size patient groups are less likely to recover than none bladder size patient groups.

Table 5 : Logistic model result with estimated odd ratio

	Logit estimates		Odds ratio estimates	
covaraites	Estimated coeff(SE)	95 % confidence	Exp(coeff)	95% confidence
Intercept	2.092(0.332)	[1.477,2.784]**	8.100	[4.379,16.187]**
Width of fistula				
<= 2centimeter				
3-5 centimeter	[-0.774(0.379)	[-1.517,-0.025]**	0.461	[0.219,0.975]**
Length of fistula				
<= 2centimeter				
3-5 centimeter	-1.060(0.424)	[-1.892,-0.223]**	0.346	[0.151,0.800]**
Bladder size				
None				
Small	-0.245(0.373)	[-0.974,0.497]	0.783	[0.378,1.644]
Fair	-1.622(0.718)	[-3.107,-0.223]**	0.197	[0.045,0.800]**
Place of delivery				
Health institution				
Home	-0.561(0.362)	[-1.274,0.152]	0.571	[0.280,1.164]

** Indicates significance at 5% level of significance

IV. Conclusion

Among all patient considered in the study during the time period the prevalence of recovery from visco vaginal fistula was 76.21%. Whereas width of fistula, length of fistula and bladder size categories were significantly associated with recovery status of the patients where weight and place of delivery of the patients associated with recovery status at 10% level of significance.

The logistic binary model was an appropriate in fitting the recovery status of the patients in comparison binary model with probit and complementary lo-log link function. Furthermore, the fitted model showed that width and length of fistula category group between three up to five centimeter and bladder size fair category group have significant effect on the recovery status of the patients.

The study also revealed that vesicovaginal fistula patients with width and length category group between three up to five centimeter were less likely to be recovered in comparison with less than or equal to two centimeter width and length of fistula category group patients.

References Références Referencias

1. Browning A. (2004) Obstetric fistula in Ilorin, Nigeria. PLoS Med 8: 369-377.
2. Lippincott W. and Wilkins (2012) Preventing obstetric fistulas in low-resource countries. women health 67(2) available from < http://women:webmd: com/tc/vaginal-fistula-topic-overview >.
3. Hilton, P. (2001) Vesicovaginal fistula new perspectives. International Journal of Gynecology and Obstetrics 82: 285-295.
4. Mikah S., Daru H. Karshima A., and Nyango D. (2011) The burden of vesicovaginal fistula in north central Nigeria. J West Afr Coll Surg. 1: 5062.
5. Dangal G., Thapa K. Yangzom K. and Karki (2013) Obstetric fistula in the developing World: An Agonising Tragedy. NJOG 8: 5-15.
6. Khaliah A., Janet M., Letu H., Elsa M., Dirk Jena and et al (2010) The role of counseling for obstetric fistula patients. Lessons learned from Eritrea, Patient Education and Counseling 80: 262-265.
7. Muleta M., Fantahun M., Tafesse B., Hamlin E., Kennedy R (2007) Obstetric fistula in rural Ethiopia. East Afr Med J 84(:52533.
8. Samanta, B., Bird L., Kuijpers M., Zimmerman A., Jarvik G. and et al (2009) Prediction of proventricular leukoplakia: Selection of hemo-dynamic features using logistic regression and decision tree algorithms. Journal of Artificial Intelligence in Medicine 46: 201 -215.
9. Dobson J. (2002) An introduction to generalized linear models Second edition. Texts in Statistical Science Series, University of British Columbia, Canada.
10. Hosmer D. and Lemeshow S. (2000) Applied Logistic Regression. Wiley-Interscience Publication, New York.
11. Brijesh S. (2011) Reporting dichotomous data using logistic regression in medical research. Journal of Epidemiology 1:111-113

Global Journals Inc. (US) Guidelines Handbook 2016

www.GlobalJournals.org

FELLOWS

FELLOW OF ASSOCIATION OF RESEARCH SOCIETY IN MEDICAL (FARSM)

Global Journals Incorporate (USA) is accredited by Open Association of Research Society (OARS), U.S.A and in turn, awards "FARSM" title to individuals.The 'FARSM' title is accorded to a selected professional after the approval of the Editor-in-Chief/Editorial Board Members/Dean.

- The "FARSM" is a dignified title which is accorded to a person's name viz. Dr. John E. Hall,Ph.D., FARSS or William Walldroff, M.S., FARSM.

FARSM accrediting is an honor. It authenticates your research activities. After recognition as FARSM, you can add 'FARSM' title with your name as you use this recognition as additional suffix to your status. This will definitely enhance and add more value and repute to your name. You may use it on your professional Counseling Materials such as CV, Resume, and Visiting Card etc.

The following benefits can be availed by you only for next three years from the date of certification:

FARSM designated members are entitled to avail a 40% discount while publishing their research papers (of a single author) with Global Journals Incorporation (USA), if the same is accepted by Editorial Board/Peer Reviewers. If you are a main author or co-author in case of multiple authors, you will be entitled to avail discount of 10%.

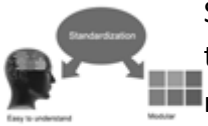
Once FARSM title is accorded, the Fellow is authorized to organize a symposium/seminar/conference on behalf of Global Journal Incorporation (USA). The Fellow can also participate in conference/seminar/symposium organized by another institution as representative of Global Journal. In both the cases, it is mandatory for him to discuss with us and obtain our consent.

You may join as member of the Editorial Board of Global Journals Incorporation (USA) after successful completion of three years as Fellow and as Peer Reviewer. In addition, it is also desirable that you should organize seminar/symposium/conference at least once.

We shall provide you intimation regarding launching of e-version of journal of your stream time to time.This may be utilized in your library for the enrichment of knowledge of your students as well as it can also be helpful for the concerned faculty members.

The FARSM can go through standards of OARS. You can also play vital role if you have any suggestions so that proper amendment can take place to improve the same for the benefit of entire research community.

As FARSM, you will be given a renowned, secure and free professional email addres with 100 GB of space e.g. johnhall@globaljournals.org. This will include Webmail, Spam Assassin, Email Forwarders,Auto-Responders, Email Delivery Route tracing, etc.



The FARSM will be eligible for a free application of standardization of their researches. Standardization of research will be subject to acceptability within stipulated norms as the next step after publishing in a journal. We shall depute a team of specialized research professionals who will render their services for elevating your researches to next higher level, which is worldwide open standardization.

The FARSM member can apply for grading and certification of standards of their educational and Institutional Degrees to Open Association of Research, Society U.S.A. Once you are designated as FARSM, you may send us a scanned copy of all of you credentials. OARS will verify, grade and certify them. This will be based on your academic records, quality of research papers published by you, and some more criteria. After certification of all your credentials by OARS, they will be published on your Fellow Profile link on website https://associationofresearch.org which will be helpful to upgrade the dignity.

The FARSM members can avail the benefits of free research podcasting in Global Research Radio with their research documents. After publishing the work, (including published elsewhere worldwide with proper authorization) you can upload your research paper with your recorded voice or you can utilize chargeable services of our professional RJs to record your paper in their voice on request.

The FARSM member also entitled to get the benefits of free research podcasting of their research documents through video clips. We can also streamline your conference videos and display your slides/ online slides and online research video clips at reasonable charges, on request.

The FARSM is eligible to earn from sales proceeds of his/her researches/reference/review Books or literature, while publishing with Global Journals. The FARSS can decide whether he/she would like to publish his/her research in a closed manner. In this case, whenever readers purchase that individual research paper for reading, maximum 60% of its profit earned as royalty by Global Journals, will be credited to his/her bank account. The entire entitled amount will be credited to his/her bank account exceeding limit of minimum fixed balance. There is no minimum time limit for collection. The FARSM member can decide its price and we can help in making the right decision.

The FARSM member is eligible to join as a paid peer reviewer at Global Journals Incorporation (USA) and can get remuneration of 15% of author fees, taken from the author of a respective paper. After reviewing 5 or more papers you can request to transfer the amount to your bank account.

MEMBER OF ASSOCIATION OF RESEARCH SOCIETY IN MEDICAL (MARSM)

The ' MARSM ' title is accorded to a selected professional after the approval of the Editor-in-Chief / Editorial Board Members/Dean.

The "MARSM" is a dignified ornament which is accorded to a person's name viz. Dr. John E. Hall, Ph.D., MARSM or William Walldroff, M.S., MARSM.

MARSM accrediting is an honor. It authenticates your research activities. Afterbecoming MARSM,you can add 'MARSM' title with your name as you use this recognition as additional suffix to your status. This will definitely enhance and add more value and repute to your name. You may use it on your professional Counseling Materials such as CV, Resume, Visiting Card and Name Plate etc.

The following benefitscan be availed by you only for next three years from the date of certification.

MARSM designated members are entitled to avail a 25% discount while publishing their research papers (of a single author) in Global Journals Inc., if the same is accepted by our Editorial Board and Peer Reviewers. If you are a main author or co-author of a group of authors, you will get discount of 10%.

As MARSM, you willbe given a renowned, secure and free professional email address with 30 GB of space e.g. johnhall@globaljournals.org. This will include Webmail, Spam Assassin, Email Forwarders,Auto-Responders, Email Delivery Route tracing, etc.

We shall provide you intimation regarding launching of e-version of journal of your stream time to time.This may be utilized in your library for the enrichment of knowledge of your students as well as it can also be helpful for the concerned faculty members.

The MARSM member can apply for approval, grading and certification of standards of their educational and Institutional Degrees to Open Association of Research, Society U.S.A.

Once you are designated as MARSM, you may send us a scanned copy of all of your credentials. OARS will verify, grade and certify them. This will be based on your academic records, quality of research papers published by you, and some more criteria.

It is mandatory to read all terms and conditions carefully.

AUXILIARY MEMBERSHIPS

Institutional Fellow of Open Association of Research Society (USA) - OARS (USA)

Global Journals Incorporation (USA) is accredited by Open Association of Research Society, U.S.A (OARS) and in turn, affiliates research institutions as "Institutional Fellow of Open Association of Research Society" (IFOARS).

The "FARSC" is a dignified title which is accorded to a person's name viz. Dr. John E. Hall, Ph.D., FARSC or William Walldroff, M.S., FARSC.

The IFOARS institution is entitled to form a Board comprised of one Chairperson and three to five board members preferably from different streams. The Board will be recognized as "Institutional Board of Open Association of Research Society"-(IBOARS).

The Institute will be entitled to following benefits:

The IBOARS can initially review research papers of their institute and recommend them to publish with respective journal of Global Journals. It can also review the papers of other institutions after obtaining our consent. The second review will be done by peer reviewer of Global Journals Incorporation (USA) The Board is at liberty to appoint a peer reviewer with the approval of chairperson after consulting us.

The author fees of such paper may be waived off up to 40%.

The Global Journals Incorporation (USA) at its discretion can also refer double blind peer reviewed paper at their end to the board for the verification and to get recommendation for final stage of acceptance of publication.

The IBOARS can organize symposium/seminar/conference in their country on behalf of Global Journals Incorporation (USA)-OARS (USA). The terms and conditions can be discussed separately.

The Board can also play vital role by exploring and giving valuable suggestions regarding the Standards of "Open Association of Research Society, U.S.A (OARS)" so that proper amendment can take place for the benefit of entire research community. We shall provide details of particular standard only on receipt of request from the Board.

The board members can also join us as Individual Fellow with 40% discount on total fees applicable to Individual Fellow. They will be entitled to avail all the benefits as declared. Please visit Individual Fellow-sub menu of GlobalJournals.org to have more relevant details.

We shall provide you intimation regarding launching of e-version of journal of your stream time to time. This may be utilized in your library for the enrichment of knowledge of your students as well as it can also be helpful for the concerned faculty members.

After nomination of your institution as "Institutional Fellow" and constantly functioning successfully for one year, we can consider giving recognition to your institute to function as Regional/Zonal office on our behalf.
The board can also take up the additional allied activities for betterment after our consultation.

The following entitlements are applicable to individual Fellows:

Open Association of Research Society, U.S.A (OARS) By-laws states that an individual Fellow may use the designations as applicable, or the corresponding initials. The Credentials of individual Fellow and Associate designations signify that the individual has gained knowledge of the fundamental concepts. One is magnanimous and proficient in an expertise course covering the professional code of conduct, and follows recognized standards of practice.

Open Association of Research Society (US)/ Global Journals Incorporation (USA), as described in Corporate Statements, are educational, research publishing and professional membership organizations. Achieving our individual Fellow or Associate status is based mainly on meeting stated educational research requirements.

Disbursement of 40% Royalty earned through Global Journals : Researcher = 50%, Peer Reviewer = 37.50%, Institution = 12.50% E.g. Out of 40%, the 20% benefit should be passed on to researcher, 15 % benefit towards remuneration should be given to a reviewer and remaining 5% is to be retained by the institution.

We shall provide print version of 12 issues of any three journals [as per your requirement] out of our 38 journals worth $ 2376 USD.

Other:

The individual Fellow and Associate designations accredited by Open Association of Research Society (US) credentials signify guarantees following achievements:

- The professional accredited with Fellow honor, is entitled to various benefits viz. name, fame, honor, regular flow of income, secured bright future, social status etc.

- In addition to above, if one is single author, then entitled to 40% discount on publishing research paper and can get 10%discount if one is co-author or main author among group of authors.
- The Fellow can organize symposium/seminar/conference on behalf of Global Journals Incorporation (USA) and he/she can also attend the same organized by other institutes on behalf of Global Journals.
- The Fellow can become member of Editorial Board Member after completing 3yrs.
- The Fellow can earn 60% of sales proceeds from the sale of reference/review books/literature/publishing of research paper.
- Fellow can also join as paid peer reviewer and earn 15% remuneration of author charges and can also get an opportunity to join as member of the Editorial Board of Global Journals Incorporation (USA)
- • This individual has learned the basic methods of applying those concepts and techniques to common challenging situations. This individual has further demonstrated an in–depth understanding of the application of suitable techniques to a particular area of research practice.

Note :

"

- In future, if the board feels the necessity to change any board member, the same can be done with the consent of the chairperson along with anyone board member without our approval.
- In case, the chairperson needs to be replaced then consent of 2/3rd board members are required and they are also required to jointly pass the resolution copy of which should be sent to us. In such case, it will be compulsory to obtain our approval before replacement.
- In case of "Difference of Opinion [if any]" among the Board members, our decision will be final and binding to everyone.

"

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 conveninet , 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 .

Before start writing a good quality Computer Science Research Paper, let us first understand what is Computer Science Research Paper? So, Computer Science Research Paper is the paper which is written by professionals or scientists who are associated to Computer Science and Information Technology, or doing research study in these areas. If you are novel to this field then you can consult about this field from your supervisor or guide.

TECHNIQUES FOR WRITING A GOOD QUALITY RESEARCH PAPER:

1. Choosing the topic: In most cases, the topic is searched by the interest of author but it can be also suggested by the guides. You can have several topics and then you can judge that in which topic or subject you are finding yourself most comfortable. This can be done by asking several questions to yourself, like Will I be able to carry our search in this area? Will I find all necessary recourses to accomplish 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.

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

save our planet

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 9755896