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

Global Journal of Medical Research: C

Microbiology and Pathology

Highlights

- Prevalence, Isolation of Bacteria
- Hemorrhagic Syndrome in Erysipelas
- Preliminary Survey of Norovirus
- Bacteriological Profile and Antibiotic

Discovering Thoughts, Inventing Future

VOLUME 16 ISSUE 1 VERSION 1.0

© 2001-2016 by Global Journal of Medical Research, USA

Global Journal of Medical Research: C
Microbiology and Pathology

Global Journal of Medical Research: C
Microbiology and Pathology

Volume 16 Issue 1 (Ver. 1.0)

Open Association of Research Society

© Global Journal of Medical Research . 2016.

All rights reserved.

This is a special issue published in version 1.0 of "Global Journal of Medical Research." By Global Journals Inc.

All articles are open access articles distributed under "Global Journal of Medical Research"

Reading License, which permits restricted use. Entire contents are copyright by of "Global Journal of Medical Research" unless otherwise noted on specific articles.

No part of this publication may be reproduced or transmitted in any form or by any means, electronic or mechanical, including photocopy, recording, or any information storage and retrieval system, without written permission.

The opinions and statements made in this book are those of the authors concerned. Ultraculture has not verified and neither confirms nor denies any of the foregoing and no warranty or fitness is implied.

Engage with the contents herein at your own risk.

The use of this journal, and the terms and conditions for our providing information, is governed by our Disclaimer, Terms and Conditions and Privacy Policy given on our website http://globaljournals.us/terms-and-condition/menu-id-1463/

By referring / using / reading / any type of association / referencing this journal, this signifies and you acknowledge that you have read them and that you accept and will be bound by the terms thereof.

All information, journals, this journal, activities undertaken, materials, services and our website, terms and conditions, privacy policy, and this journal is subject to change anytime without any prior notice.

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)

John A. Hamilton,"Drew" Jr.,
Ph.D., Professor, Management
Computer Science and Software Engineering
Director, Information Assurance Laboratory
Auburn University

Dr. Henry Hexmoor
IEEE senior member since 2004
Ph.D. Computer Science, University at Buffalo
Department of Computer Science
Southern Illinois University at Carbondale

Dr. Osman Balci, Professor
Department of Computer Science
Virginia Tech, Virginia University
Ph.D. and M.S. Syracuse University, Syracuse, New York
M.S. and B.S. Bogazici University, Istanbul, Turkey

Yogita Bajpai
M.Sc. (Computer Science), FICCT
U.S.A. Email:
yogita@computerresearch.org

Dr. T. David A. Forbes
Associate Professor and Range Nutritionist
Ph.D. Edinburgh University - Animal Nutrition
M.S. Aberdeen University - Animal Nutrition
B.A. University of Dublin- Zoology

Dr. Wenying Feng
Professor, Department of Computing & Information Systems
Department of Mathematics
Trent University, Peterborough,
ON Canada K9J 7B8

Dr. Thomas Wischgoll
Computer Science and Engineering,
Wright State University, Dayton, Ohio
B.S., M.S., Ph.D.
(University of Kaiserslautern)

Dr. Abdurrahman Arslanyilmaz
Computer Science & Information Systems Department
Youngstown State University
Ph.D., Texas A&M University
University of Missouri, Columbia
Gazi University, Turkey

Dr. Xiaohong He
Professor of International Business
University of Quinnipiac
BS, Jilin Institute of Technology; MA, MS, PhD,. (University of Texas-Dallas)

Burcin Becerik-Gerber
University of Southern California
Ph.D. in Civil Engineering
DDes from Harvard University
M.S. from University of California, Berkeley & Istanbul University

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

President Editor (HON.)

Dr. George Perry, (Neuroscientist)
Dean and Professor, College of Sciences
Denham Harman Research Award (American Aging Association)
ISI Highly Cited Researcher, Iberoamerican Molecular Biology Organization
AAAS Fellow, Correspondent Member of Spanish Royal Academy of Sciences
University of Texas at San Antonio
Postdoctoral Fellow (Department of Cell Biology)
Baylor College of Medicine
Houston, Texas, United States

Chief Author (HON.)

Dr. R.K. Dixit
M.Sc., Ph.D., FICCT
Chief Author, India
Email: authorind@computerresearch.org

Dean & Editor-in-Chief (HON.)

Vivek Dubey(HON.)
MS (Industrial Engineering),
MS (Mechanical Engineering)
University of Wisconsin, FICCT
Editor-in-Chief, USA
editorusa@computerresearch.org

Sangita Dixit
M.Sc., FICCT
Dean & Chancellor (Asia Pacific)
deanind@computerresearch.org

Suyash Dixit
(B.E., Computer Science Engineering), FICCTT
President, Web Administration and
Development , CEO at IOSRD
COO at GAOR & OSS

Er. Suyog Dixit
(M. Tech), BE (HONS. in CSE), FICCT
SAP Certified Consultant
CEO at IOSRD, GAOR & OSS
Technical Dean, Global Journals Inc. (US)
Website: www.suyogdixit.com
Email: suyog@suyogdixit.com

Pritesh Rajvaidya
(MS) Computer Science Department
California State University
BE (Computer Science), FICCT
Technical Dean, USA
Email: pritesh@computerresearch.org

Luis Galárraga
J!Research Project Leader
Saarbrücken, Germany

Contents of the Issue

i. Copyright Notice
ii. Editorial Board Members
iii. Chief Author and Dean
iv. Contents of the Issue

1. Disfibrinogenemiya and Hidden Hemolysis - Indicators of Hemorrhagic Syndrome in Erysipelas. *1-11*
2. The Histological Changes of the Skin Lesion in Diabetic Foot. *13-23*
3. Bacteriological Profile and Antibiotic Susceptibility Pattern of Blood Culture Isolates from Patients Visiting Tertiary Care Hospital in Kathmandu, Nepal. *25-31*
4. A Preliminary Survey of Norovirus and Astrovirus Antigen in Diarrheic Stools of Children in Borno State, Nigeria. *33-37*
5. Prevalence, Isolation of Bacteria and Risk Factors of Mastitis of Dairy Cattle in Selected Zones of Oromia Regional States, Ethiopia. *39-45*

v. Fellows
vi. Auxiliary Memberships
vii. Process of Submission of Research Paper
viii. Preferred Author Guidelines
ix. Index

Global Journal of Medical Research: C
Microbiology and Pathology
Volume 16 Issue 1 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

Disfibrinogenemiya and Hidden Hemolysis - Indicators of Hemorrhagic Syndrome in Erysipelas

By Elena. G. Fokina

Central Research Institute of Epidemiology, Russian Federation

Abstract- Purpose of the work: The work was aimed at the study of the changes of the system of hemostasis and of blood rheology during the progression of infection in patients with lower-limb and facial erysipelas and to validate the advisability of replacement and/or antithrombotic therapy.

Material and methods: the author studied the indices of external (time of prothrombin (PT), international normalized ratio(INR)) and internal (activated partial thromboplastin time (aPTT)) coagulation pathways, the degree of disfibrino-genemiya (thrombin time (TT), functional fibrinogen activity and D-dimer level), the amount and functional activity of the platelets (aggregation with adenosine diphosphate (ADP) and the erythrocytes (aggregation with lanthanoid (LaCl3) and protamine sulfate(PS)) in 60 cases of erysipelas. The studied indices also included endothelial dysfunction – the decrease of athrombogenicity of vascular wall endothelium (antithrombin III (AT III) and protein C) and the increase of endothelial adhesive properties (von Willebrand factor(vWf)).

Keywords: facial erysipelas, lower-limb erysipelas, hidden hemolysis, DIC-like syndrome, erythrocyte aggregation, platelet aggregation, fibrinogen, D-dimer, protein C, antithrombin - III, von Willebrand factor, haptoglobin, β – hemolytic streptococcus (β-HS).

GJMR-C Classification : NLMC Code: WA 105

Strictly as per the compliance and regulations of:

© 2016. Elena. G. Fokina. This is a research/review paper, distributed under the terms of the Creative Commons Attribution-Noncommercial 3.0 Unported License http://creativecommons.org/ licenses/by-nc/3.0/), permitting all non-commercial use, distribution, and reproduction in any medium, provided the original work is properly cited.

Disfibrinogenemiya and Hidden Hemolysis - Indicators of Hemorrhagic Syndrome in Erysipelas

Elena. G. Fokina

Abstract- Purpose of the work: The work was aimed at the study of the changes of the system of hemostasis and of blood rheology during the progression of infection in patients with lower-limb and facial erysipelas and to validate the advisability of replacement and/or antithrombotic therapy.

Material and methods: the author studied the indices of external (time of prothrombin (PT), international normalized ratio(INR)) and internal (activated partial thromboplastin time (aPTT)) coagulation pathways, the degree of disfibrinogenemiya (thrombin time (TT), functional fibrinogen activity and D-dimer level), the amount and functional activity of the platelets (aggregation with adenosine diphosphate (ADP) and the erythrocytes (aggregation with lanthanoid (LaCl3) and protamine sulfate(PS)) in 60 cases of erysipelas. The studied indices also included endothelial dysfunction – the decrease of athrombogenicity of vascular wall endothelium (antithrombin III (AT III) and protein C) and the increase of endothelial adhesive properties (von Willebrand factor(vWf)).

The comparison groups included normal volunteers (n=32) and patients with inflammation localized on the face (n=24), and the legs (n=36) at various stages of the disease (day 1–3; 4-6; 7-10; and 11-15 from the onset of the disease). These patients underwent in-hospital treatment in 2nd Moscow Clinical Hospital for the Infectious Diseases.

Results and discussion: The thesis, according to which the rate of hemorrhagic complications in lower limb erysipelas (LLE) is by 3,9 times higher than in facial erysipelas (FE), was confirmed by laboratory findings. In particular, a significant decrease of protein C was noted in patients with LLE and concomitant chronic venous insufficiency (CVI). We have found increased D-dimer and decreased α2-macroglobulin (α_2 MG) levels, suggesting potent activation of proteolytic enzymes (plasmin, matrix metalloproteinases and neutrophil elastase), which can be one of the causes of bullae, erosion and ulceration formation in the erysipelas focus on the lower limb.

The signs of intravascular (hidden) hemolysis - the decrease of haptoglobin concentration and the increase of indirect bilirubin and lactic dehydrogenase (LDH) blood levels; the changes of rheological properties of erythrocytes – the increase of deformability (aggregation with lanthanum chloride ($LaCl_3$)) and the decrease of elasticity (aggregation with protamine sulfate (PS)) – have been identified as one of the main factors for disseminated intravascular coagulation (DIC)-like syndrome in erysipelas.

Keywords: facial erysipelas, lower-limb erysipelas, hidden hemolysis, DIC-like syndrome, erythrocyte aggregation, platelet aggregation, fibrinogen, D-dimer, protein C, antithrombin - III, von Willebrand factor, haptoglobin, β – hemolytic streptococcus (β-HS).

I. Introduction

To date, clinical research focuses on the study of the relation between the inflammation and the coagulation. The dysfunction of vascular endothelium, common for these two pathological processes, is an early pathophysiological sign and an independent predictor of unfavorable prognosis of most diseases. The prevention of endothelial dysfunction of the microcirculatory bed helps to prevent and to treat many diseases.

Erysipelas is a widely spread acute infectious disease. Its development does not depend on industrial development and social security in different countries. General and local predisposing factors form an important pathogenic aspect of this condition's development. Lower limb erysipelas (LLE) is often associated with obesity, type 2 diabetes mellitus, chronic venous insufficiency (CVI) and feet and nail mycoses [1, 2]. Facial erysipelas (FE) often develops in association with otomycosis and chronic ear, nose and throat (ENT) diseases [3].

Despite modern methods of treatment, up to 10% cases of erysipelas are complicated by skin necrosis at the sites of hemorrhages and vesicles formation, by venous incompetence (periphlebitis, phlebitis and thrombophlebitis). The frequency of hemorrhagic form of erysipelas increased [4, 5]. Hence, the study of the system of hemostasis and blood rheology during the developing infectious process in patients with lower limb and facial erysipelas is of thrilling importance.

a) *Purpose of the work*

The purpose of this work was to study the changes of hemostasis and of blood rheology during infective process in patients with lower limb and facial erysipelas and to validate the advisability of replacement and/or antithrombotic therapy.

Author: M.D., Ph.D.,(infection diseases), Higher Doctorate of the Central Research Institute of Epidemiology of the Federal Service on Customers' Rights Protection and Human Well-being Surveillance. (Address: Novoguireevskaya Street, build 3a; Moscow, Russia, 111123). e-mail: e-fokina@yandex.ru

© 2016 Global Journals Inc. (US)

II. Material and Methods

a) Patient profiles

We have studied 60 patients aged 25 to 71 years. Thirty-six of them had 2nd degree lower limb erysipelas and 24 had 2nd degree facial erysipelas (24). 67% of patients had primary form of the disease. Erythematous form of erysipelas was found in 33% of cases (52% in facial erysipelas), erythematous-bullous in 15%, erythematous -hemorrhagic in 22%, and bullous-hemorrhagic in 30% of cases. Erythematous-hemorrhagic (n=11) and bullous-hemorrhagic (n=15) erysipelas affected the lower limb more frequently than the face (2 and 3 cases, respectively). The risk of hemorrhagic lesions was significantly higher in cases with local inflammatory process (78%) affecting the legs, than in face affection (20%); the odds ratio (OR) = 9,9 (2,8; 34,7).

Primary facial erysipelas diagnosed in 92% of cases was more common in women (16 women, 8 men). LLE was primary in 50% of cases, repeated in 31% and recurrent in 19% of cases. Facial erysipelas was primary in 92%, repeated in 4% and recurrent in 4% of cases. The risk of LLE recurrence was significantly higher than of the facial erysipelas (OR =5,55 (1; 51,2), p=0.009).

Gender ratio in LLE was almost equal (males-17, females - 19). Feet and nail mycosis onychomycosis were the most frequent (88%) concomitant diseases. Eleven patients had grade 2 to 4 obesity, 5 patients had subcompensated type 2 diabetes mellitus.

Skin diseases (retroaural dermatitis, streptoderma, psoriasis) were the underlying pathology in 37,5% of patients with facial erysipelas. 29% of these patients had chronic ENT diseases (otitis, tonsillitis and rhinitis). Four patients had type 2 diabetes mellitus.

The patients underwent in-hospital treatment in Erysipelas department of the 2nd Moscow Clinical Hospital for Infectious Diseases. Thirty-two patients received antibacterial monotherapy: intramuscularly (IM) Benzylpenicillin novocaine salt twice daily (1,2 mln ME per day) for 7-10 days. Another two patients received IM Cephazolin three times daily (3 g per day) for 5 days. Combined two-antibiotics therapy (IM Benzylpenicillin novocaine salt twice daily (1,2 mln ME per day) for 7-10 days and Ciprofloxacin twice daily per os (1 g per day) for 10 days) was used in 14 patients. Twelve patients got a three-antibiotics combination (IM Benzylpenicillin novocaine salt (1,2 mln ME per day) for 7 days + intravenously (IV) Ciprofloxacin (800 mg per day) for 3 days with subsequent passage to per os (1 g per day) for 10 days + IM Cephazolin three times daily (3 g per day) for 5 days). Additionally the patients received: antihistamine agents (Cetirizine dihydrochloride); topical physiotherapy (ultraviolet irradiation (UVI) therapy and low frequency current (LFC) for facial erysipelas; UVI therapy, LFC and magnetic therapy for lower limb erysipelas). The focus of LLE erysipelas was regularly treated with tanning solution of potassium permanganate. The studied patients did not receive medications capable to influence their hemostasis' state.

Mean duration of in-hospital stay was 11,9 + 4,1 days for the patients with LLE and 8,4 + 1,6 days for the patients with FE.

The hemostasis indices were studied in the beginning of the disease (days 1-3) - study point 1, during the course of the disease (days 4-6; 7-10) – points 2 and 3, and during the recovery period (days 11-15) – study point 4. Every third patient with LLE underwent the follow-up examination (5 months after the discharge). It helped to differentiate between erysipelas-induced changes and the underlying concomitant diseases.

The control group comprised 32 normal subjects aged 24 to 50 years, with equal gender distribution.

b) Statistical analysis

The results were processed on Statistica 10.0 for Windows 7.0 software. The statistically significant (reliable) differences between the groups was studied using: Mann-Whitney test for quantitative values, two independent groups; Kruskall-Wallis test for more than two independent groups; Wilcoxon test for quantitative values, related groups (pre-and post-therapy, in dynamics); chi-square test, and, if necessary, two-tailed Fisher's exact test for qualitative values. The differences evaluated with the use of parametric and non-parametric methods were considered statistically significant with confidence level >95% ($p<0.05$). In case of statistically significant inter-group differences, the odds ratio (OR) and 95 confidence interval (CI) were calculated OR (-95% CI; + 95 CI). The values in the Table are presented as Me (median) and SD – standard deviation.

III. Methodology

Laboratory investigations of biochemical indices, the parameters of hemostasis, as well as the determination of blood serum protein fractions using electrophoresis were conducted together with the specialists from the express-laboratories of the 2nd Clinical Hospital for Infectious Diseases; the determination of protein C was conducted together with the specialists from the express-laboratories of Filatov' Pediatric City Clinical Hospital. The aggregation properties of the platelets, the elasticity and the deformability of the erythrocytes were studied together with senior researchers of the laboratories of hemostasis of the clinical department of Central Research Institute of Epidemiology of Federal Service on Customers' Rights Protection and Human Well-being Surveillance (Moscow). Aggregation capacity of the platelets (using Born' method) and the erythrocytes (using Sheremetiev'

© 2016 Global Journals Inc. (US)

original technique [6]) was determined by photometric aggregometers SOLAR AP-2110 (Belarus) and BIOLA LA - 230 (RF). Platelet aggregation was induced using 2x10-5 M ADP (Reanal); erythrocyte aggregation – using 1% protamine sulfate (RF) solution and LaCl3 (RF) solution (at a dilution of 150 mg of reagent in 100 ml water.

The indices of the external (prothrombin time (PT), International Normalized Ratio (INR), Quick prothrombin index and the internal (activated partial thromboplastin time (aPTT)) coagulation pathways were turbodimetric determined on an automatic coagulometer ACL ELITE PRO (USA). The degree of disfibrinogenemiya was evaluated by the thrombin time (TT), functional fibrinogen activity (using Clauss method) and D-dimer dimension.

The endothelial dysfunction was determined by the degree of athrombogenicity of the vascular wall endothelium (antithrombin III (AT-III) and protein C levels) as well as by the adhesive properties of the endothelium (using von Willebrand factor). Blood plasma levels of AT-III was determined on ACL ELITE PRO coagulometer; protein C– on SYSMEX CA-500 coagulometer (Siemens Healthcare, USA). Von Willebrand factor (vWf) was determined by manual method (Renam reagents, RF).

Blood serum protein fractions were studied on agarose gel in HYDRASYS analyzer (Sebia, France).

The inflammatory level was estimated on the base of C-reactive protein (CRP) on HITACHI - 902 analyzer (Roche, Japan). The same analyzer was used to determine the content of haptoglobin and lactic dehydrogenase (LDH) in blood serum. According to the design of the study, we determined the substrates and the enzymes of human biochemical passport [1, 7, 8], performed clinical blood and urine analyses.

IV. Results and Discussion

During acute infection, the disturbances of hemostasis can evolve to hidden or evident signs of disseminated intravascular coagulation with threatening venous thromboses. The mechanisms of venous thromboses can be triggered by tissue lesion (in this case – infectious inflammation caused by β-hemolytic streptococcus (β-HS), the edema of the affected limb, the presence of lymphostasis and chronic venous insufficiency, the excess and/or the hyperactivity of the plasma factors. All the above factors accentuate the already existing dysfunction of the vascular wall endothelium. For this reason, a special attention is given to the study of athrombogenic and adhesive properties of the vascular wall and to the control of native anticoagulants' level (protein C and antithrombin-III) [1, 9, 10, 11].

a) Athrombogenic and adhesive properties of the vascular wall

Baseline level of protein C during the first 3 days of the disease (at admission) in patients with lower limb erysipelas (81,9±4,9%) was significantly lower than in patients with facial erysipelas (94,1±6,0%), and reliably below the control values (100±5%, $p<0,05$) as shown in Table 1.

With the decrement of the erysipelas focus, the level of protein C recovered gradually in both groups.

Protein C in patients with LLE without CVI (n=28) was 99,8±4,7% during the acute stage of the disease, and increased to 140±4,5% during the recovery stage $p<0,001$), (Figure 1). The low baseline level of protein C in lower-limb erysipelas with CVI (69,8±8,1%) did not significantly change during the recovery (79,15±4,0%, $p=0.21$) and remained refractory low during the treatment (Figure 1).

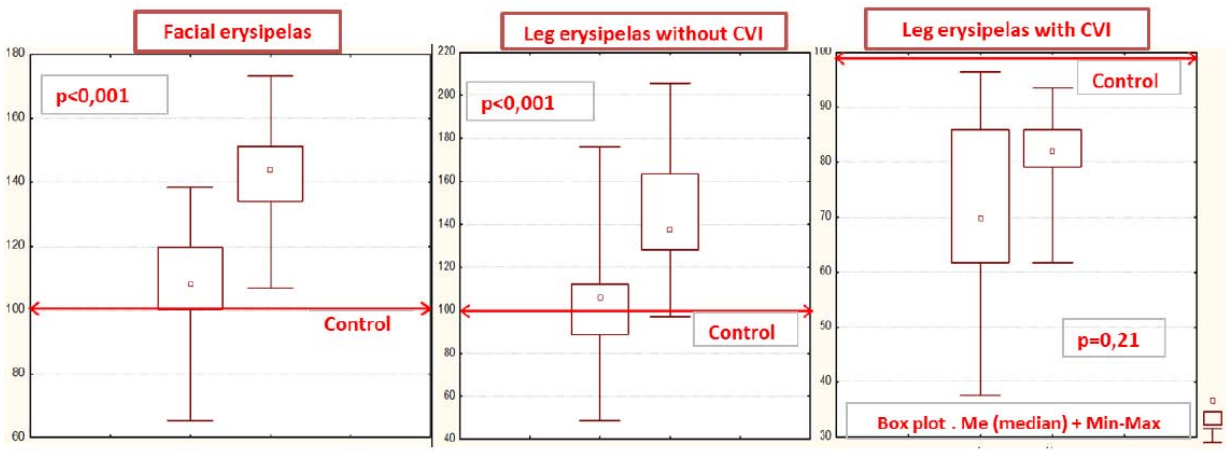

Figure 1 : Protein C in patients with facial erysipelas (1), lower-limb erysipelas without (2) and with CVI (3)
Note: Left columns - at admission (1st week of the disease), right columns –2nd-3rd weeks of the disease, recovery period (Wilcoxon test).

In facial erysipelas, the level of protein C returned to the norm during the third week of the disease (108,2±5,1% at admission and 144±4,6% at discharge; p1-4 <0,001). In LLE without CVI it recovered during the fourth week (99,8±4,7% at admission and 140±4,4% at discharge; p1-4<0,001). In LLE with CVI,

© 2016 Global Journals Inc. (US)

the level of protein C did not change: 69,8±8,1% at admission, 79,15±4,07% at discharge; p1-4=0.21), (Figure 1).

In the presence of endothelial dysfunction, the level of protein C in certain patients significantly increased during recovery - from study point 1 to study point 4 (from 49,7 to 112%, increase by 125%; from 48,9 to 110,7% , increase by 126%; from 65,5 to 119,7% increase by 83%). This is a good example of the adaptation mechanism (Figure 2).

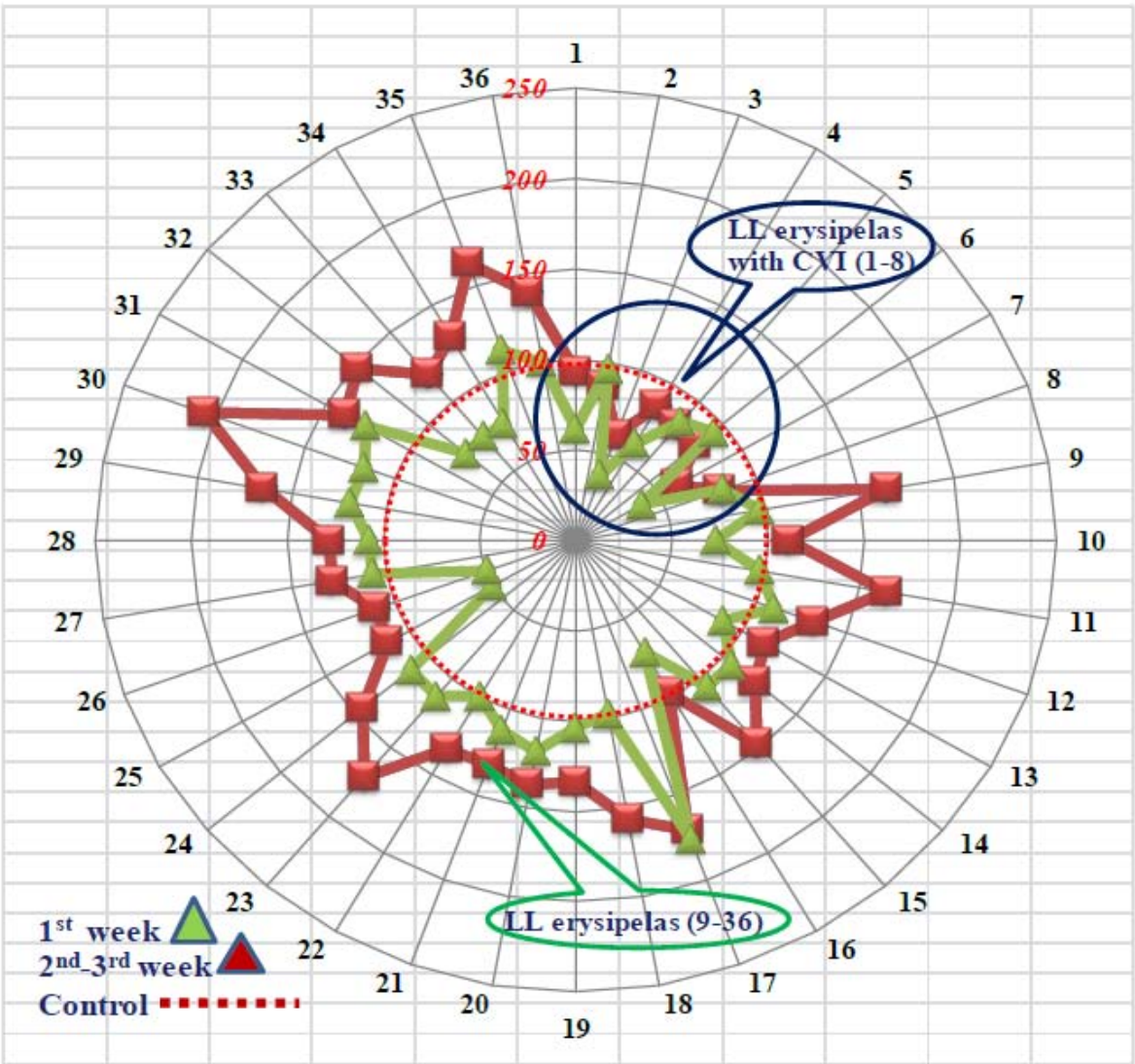

Figure 2 : Changes of protein C levels in patients with lower limb erysipelas

Note: 1-8 with CVI, 9-36 without CVI, LL - lower limb

The recovery in LLE without CVI (and with positive dynamics of protein C level) was more favorable than in LLE with concomitant CVI. According to some authors [1, 4, 9, 10, 12], CVI leads to the prolongation of the healing time of erysipelas focus and of the recovery. We have shown that in the presence of normal protein C (100±5%), the chances for the favorable course of LLE are significantly higher (OR=2,89 (0,15; 55)), than in erysipelas with low protein C and concomitant CVI.

We determined also the level of another, not less important natural anticoagulant – the antithrombin - III. According to the literature [13, 14], the thrombosis (strokes, infarctions) develop when AT-III level is 80-90%. AT-III deficit occurs in the presence of clinical signs of disseminated intravascular coagulation and the multiorgan failure syndrome.

AT-III deficit was more pronounced in LLE, than in facial erysipelas (Table №1). The baseline values of AT-III in patients with LLE (87,6±2,5%) were significantly lower than in facial erysipelas (91,8±2,5%), and lower than the control values (p<0,05). AT-III level did not recover up to the discharge (Table №1). Bigger AT-III deficit seen in patients with LLE can explain higher incidence of hemorrhagic forms of erysipelas in this group of patients in comparison with facial erysipelas (78% in LLE and 22% in FE). Besides, AT-III (as well as

© 2016 Global Journals Inc. (US)

fibrinogen, CRP, α1-acid glycoprotein) is an acute-phase protein. Our previous studies had shown that the level of acute-phase proteins in patients with lower limb erysipelas was higher than in patients with facial erysipelas [8].

The concentration of α2-macroglobulin decreased by 25%. The minimal level of α2–MG (an inhibitor of various proteases, including plasmin) was seen at the end of the 1st week of the disease: 3,78±0,16% in LLE and 3,96±0,16% in FE.

β-hemolytic streptococcus produces pathogenicity factors as streptokinase, hyaluronidase, streptodornase, etc. These factors destroy the protective level of heparansulfate, lining the vascular endothelium. This is accompanied by an increase of prothrombogenic properties of the vascular wall, the release of von Willebrand factor (vWf) and the decrease of AT-III activity. We noted an increase of vWf (187% to 220%) during 1st week of the disease in all patients with erysipelas. With the extinction of the inflammatory focus, high values of vWf tended to decrease, however they never reached normal values (Table 1).

Thus, the results of the study of endothelial markers in erysipelas patients are suggestive of a compromised endothelium-related hemostasis regulation. It concerned not only antithrombotic (decreased levels of protein C and AT- III), but also adhesive characteristics (high level of vWf). The deficit of natural anticoagulants was more pronounced in LLE cases. The refractivity of protein C in patients with LLE and CVI is a diagnostic marker of CVI and indication for vascular replacement therapy [13, 14].

It is known, that the body uses natural anticoagulants for the isolation (delimitation) of infectious inflammation area. The decrease of anticoagulants' concentration «opens the gate» for the generalization of infectious inflammation [11, 15, 16, 17].

b) Changes in plasma hemostasis and disfibrinogenemiya

We have found the following shifts in the indices of the external (prothrombin time, prothrombin index, INR) and the internal (aPTT) coagulation pathways, in the degree of disfibrinogenemiya (thrombin time, functional platelets' activity and D-dimer level) in patients with erysipelas (Table 2):

- The activation of coagulation cascade during the acute stage of the disease (decreased TT in facial and lower-limb erysipelas during the days 1-3 of the disease in comparison with the control ($p<0,05$). It means the active processes of thrombin and fibrin formation in the blood flow;
- The activation of the external coagulation pathway (increased PT time in lower- limb erysipelas in comparison with the control ($p=0,033$), decreased prothrombin index and increased INR);
- The activation of the internal coagulation pathway (the increase of aPTT at admission and during recovery in LLE. In facial erysipelas, the baseline aPTT was below the control ($p < 0,05$) and increased by the end of the 1st week of the disease ($p < 0,025$);
- The disfibrinogenemiya (decreased TT with the activation of fibrin polymerization process and the appearance of a great amount of D-dimers in the patient's blood (Table № 3). The fibrinogen level in LLE was higher than in FE: 8,0 ±2,3 g/l vs 5,9±1,8 g/l ($p=0,008$) in 1- 6th days and 6,6 ±2,4 g/l vs. 4,3 ±1,0 g/l ($p<0,0001$) in 7-15th days. Fibrinogen is acute-phase protein, as well as: AT-III, CRP, presepsin, procalcitonin, α - tumor necrosis factor, interleukin - 6 [16, 14].

Hence, the disfibrinogenemiya and the activation of coagulation cascade were higher in lower limb erysipelas (see above). The peak changes occurred at 4-6th days (study point 2), (Table 3).

The initial fibrinogen level in LLE (7,7±0,38 g/l) was by 18% higher than in FE (6,53± 0,49 g/l). D-dimer level in lower-limb erysipelas (399±46 ng/ml) was more than twofold higher in comparison with FE patients (160,2±41 mg/ml) and by 27 times (!) higher than in control group (14,5+3,18 ng/ml, $p<0,001$).

The documented differences in D-dimer level allowed us to conclude that the processes of intravascular coagulation in lower limb erysipelas are more intense than in facial erysipelas. The presence of the clots of polymerized fibrin is a necessary condition for the increase of D-dimer level, as the process of plasminogen transformation to plasmin takes place inside these clots [5, 11, 13].

The increase of D-dimers and the decrease (expenditure) of α2-macroglobulin also are suggestive of a potent local activation of proteolysis enzymes (plasmin, matrix metalloproteinases and neutrophil elastase). These enzymes destroy the extracellular matrix and induce the process of erosions, ulcers and necrosis formation in the area of infectious inflammation.

Starting from the 2nd week of disease, with the improvement of patients' condition, the levels of acute-phase proteins (fibrinogen, CRP, α1-antitripsin (α1-AT) etc.) decreased (Table 3).

The presence of disfibrinogenemiya, the activation of external and internal coagulation pathways, as well as the increase of acute-phase proteins suggest a close relation between the systemic inflammatory response and the compromised hemostasis there.

The risk of severe (erythematous-bullous, erythematous-hemorrhagic, bullous-hemorrhagic) forms of LLE was significantly higher compared with FE (OR= 4,9 (1,5; 16)).

© 2016 Global Journals Inc. (US)

c) Platelets' aggregation activity and erythrocytes' rheological properties

In normal settings, the circulating platelets do not interfere with the internal surface of the vessel, covered by a thin layer of heparansulfate, that confers athrombogenic and anti-adhesive properties to vascular endothelium. The vascular wall injury results in the exposition of subendothelium components, mainly collagen, into the blood flow. In case of the participation of vWf (interaction with the platelet GP1b receptors) and of fibrinogen (interaction with the platelet GPIIb/IIIa receptors), the processes of platelet adhesion and aggregation are significantly enhanced [**Ошибка! Источник ссылки не найден.**, 15].

In our study, the ADP-induced platelet aggregation was minimal at 1-3th days: 41,7 ±4% in facial erysipelas and 64,8 ±3,9 % in LLE ($p<0.05$), which is statistically lower in comparison with platelets' functional activity in normal subjects (76±3,1%, $p<0.05$). At 11-15th days of the disease, the platelets' functional activity tended to recover (71,7±3,4% in LLE and 59±3,2% in FE). The number of platelets increased in both groups: from 224±10x109/l to 408±21x109/l (p=0,002) in lower limb erysipelas and from 234±30 x109/l to 317±29x109/l (p=0,04) in facial erysipelas. Hence, the platelets' amount and their functional activity in erysipelas occurred on the 2nd week of disease.

Herewith, the initially normal amount of erythrocytes at admission (study point 1) decreased by the days 7-10 of the disease (study points 2 and 3). The number of cells decreased by 7% of the initial value (from 4,7 to 4,39 x1012/l) in facial erysipelas and by 5% (from 4,6 to 4,37 x1012/l) in lower limb erysipelas. Simultaneously, erythrocyte sedimentation rate (ESR) rose to the maximum: 53,7±8,5 mm/hour in lower limb erysipelas and 26,3±5,8 mm/hour in facial erysipelas (p=0.006).

Earlier, we have found an increase of indirect bilirubin and LDH blood levels in patients with erysipelas. Together with the decreased of haptoglobin found in this study (3,86±,26 g/l in FE and 3,79±0,3 g/l in LLE ($p<0,05$ with the control), it suggests the presence of hidden hemolysis [7]. Further changes of haptoglobin level confirm this conclusion. The level of this protein increased by 153 % of the initial values in facial erysipelas and by 61% — in LLE (Figure 3).

Hence, intravascular (hidden) hemolysis is one of the leading pathogenetic mechanism of DIC-like syndrome. This syndrome is often described as a clinically unapparent (local) disseminated intravascular coagulation. In our study, we have seen the transformation of DIC-like syndrome to the classic DIC in three cases - 5% of 60 studied patients [8].

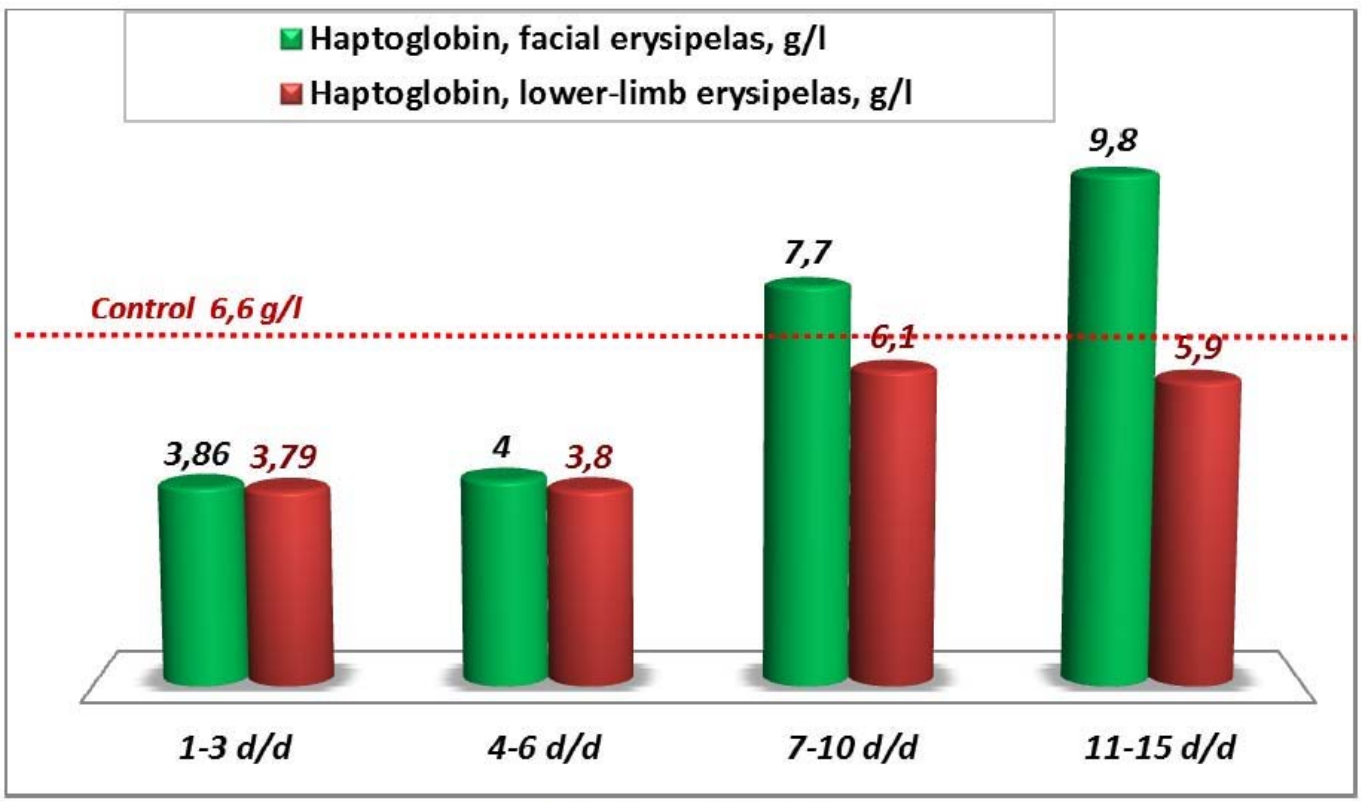

Figure 3 : Changes of haptoglobin level (g/l) in facial and lower limb erysipelas at 1st-2nd week of the disease

Note: d/d - days of the disease

We studied also the changes of rheological properties of erythrocytes – their elasticity (the aggregation with protamine sulfate) and deformability (the aggregation with lanthanum chloride) in erysipelas.

© 2016 Global Journals Inc. (US)

The degree of blood cells' elasticity and deformability in normal subjects is almost equal: 62±4,9 % for PS and 66,4±4,2 % for LaCl3.

In erysipelas, the cells' elasticity decreased twofold (aggregation with PS), while the deformability (aggregation with LaCl3) increased by 37% (Table **4**). The aggregation with two types of inductors (LaCl3 and PS) was recorded simultaneously on aggregometer «BIOLA». The aggregates were 3,6 times bigger in size and 7,8 times higher in aggregate's degree on LaCl3 compared with PS.

After addition of LaCl3, the erythrocytes of erysipelas patients interacted faster (3-5 minutes) and quickly precipitated as large conglomerates [1, 8].

Some authors also described the changed conformation properties of the erythrocytes' membrane, the cells' form transformation from concave-discoid to spherical. Our experiments showed that with the addition of LaCl3 to the erythrocytes of erysipelas patients, cytoarchitectonics of cellular membranes is disturbed. It leads to fast adhesion between the cells and to the formation of cell conglomerates. The aggregation with LaCl3 helps to reproduce the picture of erysipelas-associated hidden hemolysis and increased erythrocyte "frailness" in vitro.

The mechanism of protamine sulfate action is different. It does not induce conformational rebuilding of the erythrocytes' membrane. With the loss of negative charge of the membrane (preventing cells adhesion to each other), the erythrocytes sediment and form the coin columns. The aggregates on the PS look smaller and softer, and the aggregation time is slower (≥10 minutes) [1, 8].

The found differences in erythrocytes' elasticity and deformability persisted during the recovery period. The high "frailness" of erythrocyte membrane persisted up to the end of the 2nd week, and its low "plasticity"- up to the discharge (Figure 4).

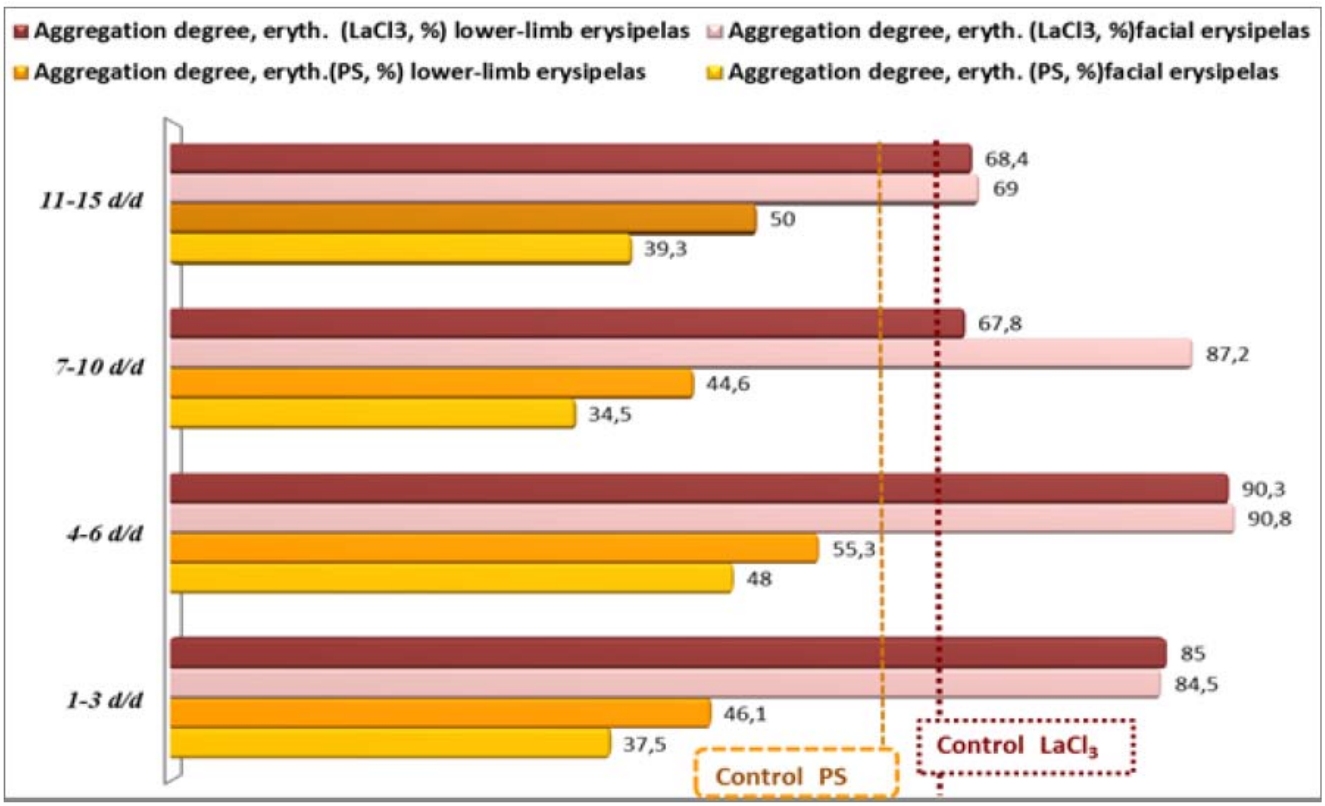

Figure 4 : Erythrocytes' deformability (LaCl3) and elasticity (PS) in aggregation tests in facial and lower limb erysipelas

Note: d/d — days of the disease

One has to note, that the erythrocytes play not only the role of oxygen transporter in the blood flow. They also serve as a potent buffer of the system of hemostasis and as a second, in order of importance (after albumin), detoxification barrier. The body uses the erythrocyte pool for supplementary refilling of protein deficit in the blood flow [7]. The discoid form allows the erythrocytes to perform their functions with maximal effectiveness. For this reason, the recovery of erythrocytes' elasticity is of clinical interest for certain therapeutic practices.

V. Conclusion and Recommendations

1. Laboratory studies confirmed that clinical hemorrhagic complications are by 3,9 times more

© 2016 Global Journals Inc. (US)

common in lower limb erysipelas than in facial erysipelas.

2. The results of determination of the markers of hemostatic endothelial function in erysipelas patients suggest the disturbances of endothelium-related regulation of hemostasis, as judged by antithrombotic (decreased levels of protein C and of AT- III), as well as by adhesive indices (high level of von Willebrand factor).
3. Increased D-dimer and decreased alpha-2-macroglobulin levels suggest potent activation of the system of proteolysis enzymes (plasmin, matrix metalloproteinases and neutrophil elastase). This is pathogenetically associated with the formation of bullae, erosions and ulcers in the erysipelas focus.
4. Low level of protein C, persisting during standard therapeutic procedures, is not only a laboratory indicator of chronic venous insufficiency in lower limb erysipelas, but also serves as a marker of the development of DIC-like syndrome.
5. Documented evidence of protein C deficit can be an indication for the consideration of antithrombotic therapy. Maximally early start of the replacement therapy (with protein C products) can contribute to the delimitation of the inflammation area and to the decrease of the severity of local proteolysis reactions.
6. We found the signs of intravascular (hidden) hemolysis and of disturbed rheological properties of erythrocytes – their increased deformability (aggregation with lanthanum chloride) and decreased elasticity (aggregation with protamine sulfate). The disturbed erythrocytes elasticity is an indication for the supplementation of standard erysipelas therapy with the agents contributing to the increase of erythrocytes' plasticity and to the improvement of blood rheology (Pentoxifylline).
7. Clinically important risk of hemorrhagic complications in lower limb erysipelas is higher, than in facial erysipelas, OR = 9,88 (2,81; 34,7).

VI. Acknowledgement

This study was designed and conducted without funding or sponsorship. No reported conflict of interest by the author.

Table 1 : Changes in protein C, AT-III and vfW levels in facial and lower-limb erysipelas (Me ±SD)

Changes by days/Index	Facial erysipelas (n=24)			Lower-limb erysipelas (n=36)		
	Protein C, %	AT-III,%	vfW,%	Protein C, %	AT-III,%	vfW,%
Days 1-3 of the disease (point 1)	94,1±6,0*,**	91,8±2,5*	187,0±4,2**	81,9±4,9*,**	87,6±2,5*	190±2,0**
Days 4-6 of the disease (point 2)	119,6±3,1*,**	96,4±0,8**	183,0±2,7**	103,0±3,2*,**	91,3±1,7*,**	187,6±2,9*
Days 7-10 of the disease (point 3)	129,0±6,4*	91,4±2,2*	175,6±6,2*	134,5±4,7*	89,6±1,5*	180,0±2,8*
Days 11-15 of the disease (point 4)	153,0±4,4*	93,0±1,1*	174,8±4,4*	139,0±6,7*	89,4±3,2*	176,0±3,1*
Follow-up (in 5 months)	_____	_____	_____	106,4±10,2	93,0±1,2*	167,0±3,7*
Normal subjects (n=32)	100±5,0	97,3±0,38	150,4±3,9	100,0±5,0	97,3±0,38	150,4±3,9

*Note: *— significant difference with the control value, **— reliable differences between the groups (p<0,05)*

© 2016 Global Journals Inc. (US)

Table 2 : Changes in plasma hemostasis in facial and lower-limb erysipelas (Me ±SD)

Changes by days/Index	Facial erysipelas (n=24)					Lower-limb erysipelas (n=36)				
	PT, sec	INR	Prothrombin, %	TT, sec	aPTT,sec	PT, sec	INR	Prothrombin, %	TT, sec	aPTT,sec
Days 1-3 of the disease (point 1)	12,2±0,7*,**	1,27±0,04*	79,2±4,5*,**	12,2±0,5*,**	28,5±1,3*,**	15,4±0,5*,**	1,47±0,04*,**	59,3±2,8*,**	10,9±0,36*,**	39,5±1,4*,**
Days 4-6 of the disease (point 2)	14,9±0,6*	1,42±0,05*	68,7±4,3*	12,7±0,7*,**	45,1±2,7*,**	15,1±0,4*	1,47±0,04*	61,3±3,8*	11,9±0,5*	38,5±1,7*,**
Days 7-10 of the disease (point 3)	12,2±0,7*,**	1,27±0,04*	79,2±4,5*,**	12,2±0,5*,**	28,5±1,3*,**	15,4±0,5*,**	1,47±0,04*,**	59,3±2,8*,**	10,9±0,36*,**	39,5±1,4*,**
Days 11-15 of the disease (point 4)	12,2±0,7	1,16±0,03	79,4±4,8*	12,2±0,3*,**	37,3±3,0*,**	13,3±0,25*,**	1,31±0,03*,**	73,3±2,8*,**	12,1±0,26*	37,4±1,3*,**
Follow-up (in 5 months)	–	–	–	–	–	11,2±0,3	1,1±0,02	93,2±2,9	13,4±0,15	30,4±1,0
Normal subjects (*n* =32)	10,9±0,14	1,11±0,02	98,2±1,8	14,6±0,26	33,7±0,66	10,9±0,14	1,11±0,02	98,2±1,8	14,6±0,26	33,7±0,66

Note: PT — Prothrombin time, INR — International Normalized Ratio, TT — Thrombin time, aPTT — activated Partial Thromboplastin time;— significant difference with the control value, **— reliable differences between the groups (p<0,05).*

Table 3 : Signs of disfibrinogenemiya, (Me ±SD)

Changes by days/Index	Facial erysipelas (*n* =24)				Lower-limb erysipelas (*n* =36)			
	Fibrinogen, g/l	D-dimer, ng/ml	CRP, mg/l	α_1-AT +α_1-acid GP, %	Fibrinogen, g/l	D-dimer, ng/ml	CRP, mg/l	α1-AT +α1-acid GP, %
Days 1-3 of the disease (point 1)	6,53±0,49*,**	160,2±4*,**	126±14*	3,63±0,2*	7,7±0,38*	399±46*,**	126±8,5*	3,79±0,29*
Days 4-6 of the disease (point 2)	5,62±0,47*,**	164±2*,**	44,5±7,1*,**	3,34±0,08*	6,75±0,42*	371±88*,**	117±13*,**	4,2±0,3*,**
Days 7-10 of the disease (point 3)	5,0±0,33*	147,7±27*,**	1,2±0,2*	3,22±0,14 *	5,3±0,17*	381±43*,**	45,7±15*	3,45±0,2*
Days 11-15 of the disease (point 4)	4,2±0,2*,**	88,6±12,7*,**	2,0±0,3*,**	2,98±0,13 *	5,6±0,4*,**	467±46*,**	9,4±3*,**	3,2±0,24*
Follow-up (in 5 months)	____	____	____	____	5,1±0,6*	202,6±5*	1,7±0,4*	2,5±0,2
Normal subjects (*n* =32)	2,96±0,09	14,5±3,18	0,6±0,2	2,6±0,1	2,96±0,09	14,5±3,18	0,6±0,2	2,6±0,1

Note: CRP — C reactive protein, α1-AT — α1-antitrypsin, α1-acid GP — α1-acid GP glycoprotein;
**— significant difference with the control value, **— reliable differences between the groups (p<0,05).*

© 2016 Global Journals Inc. (US)

Table 4 : Platelets' number and aggregation activity and erythrocytes' rheological properties in facial and lower-limb erysipelas, (Me ±SD)

Changes by days/Index	Platelets, $x10^9$/l	ADP-aggregation degree: platelets, %	Erythrocytes, $x10^{12}$/l	ESR, mm/hour	$LaCl_3$-aggregation degree: erythrocytes, %	PS - aggregation degree: erythrocytes, %	Haptoglobin, g/l
Facial erysipelas (n=24)							
Days 1-3 of the disease (point 1)	234±30,0	41,7±4*,**	4,5±0,08*	20,4±1,8*,**	84,5±6,4*,**	37,5±3,7*,**	3,86±0,26*,**
Days 4-6 of the disease (point 2)	249±9,4	67,2±5,1	4,7±0,08	18±2,8*,**	90,8±5,3*,**	48±3,1*,**	4,0±0,4*,**
Days 7-10 of the disease (point 3)	296±39	47±2,6*,**	4,39±0,08	26,3±5,8*,**	87,2±3,6*,**	34,5±1,7*,**	7,7±1,2*,**
Days 11-15 of the disease (point 4)	317±29	59±3,2*,**	4,3±0,2	16,5±2,3*	69±7,3	39,3±3,9*,**	9,8±,4*,**
Lower-limb erysipelas (n=36)							
Days 1-3 of the disease (point 1)	224±10	64,8±3,9*,**	4,7±0,06*	33,5±3,9*,**	85±4,5*,**	46,1±2,7*,**	3,79+0,3*,**
Days 4-6 of the disease (point 2)	253±14	68,2±4,3	4,6±0,08	35,2±3,7*,**	90,3±4,8*,**	55,3±5,0	3,8+0,4*,**
Days 7-10 of the disease (point 3)	314±12,8	53,7±3,0*,**	4,37±0,14	53,7±8,5*,**	67,8±5*,**	44,6±3,4*,**	6,1+0,8
Days 11-15 of the disease (point 4)	408±21	71,7±3,4*	4,5±0,1	33,7±6,4*,**	68,4±4,9	50,0±6,0*,**	5,9+0,6
Follow-up (in 5 months)	—	52,7±5,5*	—	—	63,0±6,4	36,7±3,4*,**	5,4+0,8
Normal subjects (n=32)	250+5,2	76±3,1	4,5±0,13	≤ 10	66,4±4,2	62±4,9	6,6±0,5

Note: ESR - erythrocyte sedimentation rate,— significant difference with the control value, **— reliable differences between the groups (p<0,05)*

References Références Referencias

1. Fokina E.G., Rosly I.M., Potekaeva S.A. Laboratory evaluation of erysipelatous imflammation // Epidemiologia i infekcionnye bolezni. Aktualnye voprosy (Epidemiology and infectious diseases. Thrilling questions). Moscow–2014; 1: 28–32. (in Russian).
2. Denis F., Martin C., Ploy M.C. Erysipelas: microbiological and pathogenic data // Ann. Dermatol. Venereol. 2001; 128: 317–326.
3. Morozova S.V., Timurzieva A.B. Professional view of the problem of external otitis// iDoctor: Moscow–2015; 3: 8–11. (in Russian).
4. Frolov A.P. Etiopathgenetic particularities of the development of necrotic form of erysipelas, its prediction and principles of complex therapy// Synopsis of PhD thesis. Irkutsk – 2003 (in Russian)
5. Mitrofanova M.Yu. Disturbances of hemostasis and vascular endothelial function in patients with erysipelas// Synopsis of PhD thesis. Moscow– 2010 (in Russian).
6. Sheremetiev Yu.A., Makin G.I., Suslov F.Yu. A method for the registration of the changes of surface erythrocyte charge (lanthanum chloride)// Federal Service for Intellectual Property. Russian Federation Patent № 2027188, Moscow– 1995 (in Russian).
7. Papachristodoulou D., Snape A., Elliott W.H., Elliott D.C. Biochemistry and Molecular Biology. 5th ed. Oxford: Oxford university press, 2014. 592 p.
8. Fokina E.G. Some particularities of primary erysipelas under present-day conditions.// Terapevtichesky arkhiv (Therapeutic archives) Moscow–2014; 11: 70–77. (in Russian).
9. Castellino F.J., Ploplis V.A. The protein C pathway and pathologic processes // J. Throm. Haemost. 2009; 7 (1): 140–145.
10. Dahlback B. Resistance to activated protein C as risk factor for thrombosis: molecular mechanism, laboratory investigation and clinical managements// Semin. Hematol. 1997, 34(3); 217–234.
11. Esmon C.T. Role of coagulation inhibitors in inflammation. Thromb. Haemost. 2001; 86: 51–56.

© 2016 Global Journals Inc. (US)

12. Ratnikova L.I., Dubovikova T.A. Evaluation of the state of vascular platelet hemostasis in patients with hemorrhagic forms of erysipelas// Zhurnal infectologii (Journal of Infectology). Moscow– 2012; 4 (1): 53–57 (in Russian).
13. Bockeria L.A., Klimovich L.G., Potekhina A.V./ Mechanisms of coagulation inhibitors involvement into the development of inflammatory reaction and perspective trends of anticoagulation therapy// Klinicheskaya fisiologia krovoobraschenia (Clinical physiology of the circulation) Moscow. 2004; 1: 46–55 (in Russian).
14. Bernard G.R., Vincent J.L. Efficacy and safety of recombinant human activated protein C for severe sepsis. N. Engl. J. Med. 2001; 344: 699–709.
15. Coughlin S.R. Protease-activated receptors in vascular biology. Thromb. Haemost. 2001; 86: 298–307.
16. Esmon C.T., Taylor F.B. Inflammation and coagulation: Linked processes potential regulated through a common pathway mediated by protein C. Thromb. Haemost. 1991; 66: 160–165.
17. Chen D. and Dorling A. Critical roles for thrombin in acute and chronic inflammation // J. Throm. Haemost. 2009; 7 (1): 122–126.
18. Sridharan P., Chamberlain R.S. The efficacy of procalcitonin as a biomarker in the management of sepsis: slaying dragons or tilting at windmills? Surg. Infect. (Larchmt). 2013; 14(6): 489—511.
19. Kile B.T. The role of the intrinsic apoptosis pathway in platelet life and death // J. Throm. Haemost. 2009; 7 (1): 214-217.

© 2016 Global Journals Inc. (US)

This page is intentionally left blank

© 2016 Global Journals Inc. (US)

Global Journal of Medical Research: C
Microbiology and Pathology
Volume 16 Issue 1 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 Histological Changes of the Skin Lesion in Diabetic Foot

By Muna Z. Al-Hamdany, Professor Dr. Abduljabbar Y. Al-Hubaity
& Modhar S. Al-Omary
University of Mosul, Iraq

Abstract- Objective: To investigate the histological changes in the skin tissue covering the area surrounding the ulceration of the diabetic foot.

Patients & Methods: The study was performed on 30 patients who were classified into 3 groups 10 patient in each. Group I is the control group have no diabetes, group II with diabetes mellitus type I, and Group III patients with diabetes mellitus type II. All the diabetic patients showed various degrees of skin lesions in the foot. Specimens of skin tissue were obtained from the area surrounding the diabetic ulcer from Al- Jumhuri Teaching Hospital and the histological analysis was performed in the Department of Anatomy, College of Medicine, University of Mosul from November 2015 to June 2016. The specimens were processed for the standard histological examination using Hematoxylin and Eosin, and Orcein- VanGieson stains.

Keywords: diabetic foot, microangiopathy, neuropathy.

GJMR-C Classification : NLMC Code: QW 4

THEHISTOLOGICALCHANGESOFTHESKINLESIONINDIABETICFOOT

Strictly as per the compliance and regulations of:

© 2016. Muna Z. Al-Hamdany, Professor Dr. Abduljabbar Y. Al-Hubaity & Modhar S. Al-Omary. This is a research/review paper, distributed under the terms of the Creative Commons Attribution-Noncommercial 3.0 Unported License http://creativecommons.org/ licenses/by-nc/3.0/), permitting all non-commercial use, distribution, and reproduction in any medium, provided the original work is properly cited.

The Histological Changes of the Skin Lesion in Diabetic Foot

Muna Z. Al-Hamdany [α], Professor Dr. Abduljabbar Y. Al-Hubaity [σ] & Modhar S. Al-Omary [ρ]

الخلاصة- **هدف البحث** : تهدف هذه الدراسة الى التعرف على التغيرات النسيجية في سن الجلد المحيط بقدم السكري. **المرضى و الطرق العمل**: اجريت هذه الدراسة على ثلاثين مريض تم تقسيمهم الى ثلاث مجاميع عشرة في كل مجموعة.المجموعة الاولى هي مجموعة الاصحاء والمجموعة الثانية يعانون من مرض السكري النوع الاول والمجموعة الثالثة يعانون من مرض السكري النوع الثاني . جميع مرضى السكري في هذه الدراسة لديهم تقرحات من مختلف الاحجام.تم اخذ النماذج من الجلد المحيط بالقدم السكري وتم تجميعها من مستشفى الجمهوري التعليمي بين اذار الى ايلول ٢٠١٥ بمفهوم الانسجة الطبية في كلية الطب الموصل في الفترة من ٢٠١٦ تم تحضير النماذج للفحص النسيجي باستخدام صبغة الهيماتوكسيلين وصبغة Van-Gieson. **النتائج** : تظهر النتائج وجود تقرن في البشرة مع تسرب بسيط في الخلايا الالتهابية في البشرة، انعدام او الحلال في الغدد العرقية مع تقرن في البشرة، وجود مناطق نخرية في الطبقة الدمية الاعوية الموافية لوجود الخلايا البلعمية. اما الشرايين الدقيقة في الطبقة الدمية تظهر تليف في الجدران الوعائية والشرايين العضلية متوسطة الحجم تظهر تكبير والشرايين الكبيرة تحتوي على احشاء في الطبقة الانسية من الاوعية الدموية. اما الاعصاب المحيطية فقد تظهر انحلال واضح في الخلايا الشوانية. **الاستنتاجات**: التغيرات الوعائية الدموية المشرعة في الاوعية الدموية في الجلد المحيط بالقدم السكري تظهر في البشرة والشرايين الكبيرة والمتوسطة الحجم والشرايين الكبيرة تحتوي على احشاء في الطبقة الانسية. اما التغيرات النسيجية الاعصاب المحيطية فتظهر تغيرات خطيرة في الاعصاب المحيطية وهف السكري تقرحات في الجلد المحيط بالقدم السكري وهف الشمل طبقتي البشرة والاوعية الدموية. اما التغيرات النسيجية فاشار الى امكانية تفرعمو تفرعات الاوعية الدموية في الجلد المحيط بالقدم السكري يتيح تفرعم اكثر والتي تسبب انسداد الاوعية الدموية في الاطراف السفلية. **الخلاصة**: تغيرات الاوعية الدموية في القدم السكري، مرض الاوعية الدموية،مرض الاعصاب المحيطية هذه الحالة المرضية تلقي الضوء على الخلفية المرضية والخلفية الباثولوجية لهذه الحالة.

Abstract- Objective: To investigate the histological changes in the skin tissue covering the area surrounding the ulceration of the diabetic foot.

Patients & Methods: The study was performed on 30 patients who were classified into 3 groups 10 patient in each. Group I is the control group have no diabetes, group II with diabetes mellitus type I, and Group III patients with diabetes mellitus type II. All the diabetic patients showed various degrees of skin lesions in the foot. Specimens of skin tissue were obtained from the area surrounding the diabetic ulcer from Al-Jumhuri Teaching Hospital and the histological analysis was performed in the Department of Anatomy, College of Medicine, University of Mosul from November 2015 to June 2016. The specimens were processed for the standard histological examination using Hematoxylin and Eosin, and Orcein-VanGieson stains.

Results: The skin sections were dominated by the presence of hyperkeratosis in the epidermis with regular acanthosis in addition to dense chronic inflammatory cells infiltration in the dermis, absence or degeneration of the sweat glands, perivascular lymphocytic infiltration, focal areas of necrosis and melanin pigmentation in the dermis. The large arterioles and arteries of muscular type revealed fibrous tissue deposition at the level of media while the peripheral nerves showed an obvious degeneration of Schwann cells.

Conclusions: The vascular changes in the diabetic foot appears in the microcirculation level (capillaries) then involves arterioles and arteries of muscular type and were accompanied by morphological changes of the peripheral nerves. The morphological changes in the skin surrounding the diabetic foot ulcer involves both the epidermis and dermis. The identification of histological and vascular alterations in the diabetic foot allows more knowledge related to the pathogenesis and pathological background of this condition.

Keywords: diabetic foot, microangiopathy, neuropathy.

I. Introduction

Diabetic foot is the most common complication of diabetes. The ulceration is frequently associated with peripheral neuropathy which is a well-known cause of morbidity and even mortality in the diabetic patients[1]. The incidence is rising as a result ageing and increased risk factors for atherosclerosis such as smoking and obesity commonly associated with diabetes[2]. The diabetic foot lesions involves a wide range of structural changes affecting the nerves in the form of autonomic and motor neuropathy, blood vessels as diabetic macro and microangiopathy, joint and bone lesions of the sole, and skin and nail lesions[3]. The exact mechanism underlying the pathogenesis of diabetic ulceration is not well known, many mechanisms have been proposed even there may be a genetic influence which increase the susceptibility to such complications[4]. However, peripheral neuropathy is the major cause combined with arterial insufficiency caused by atherosclerotic occlusion of the tibioperoneal arteries[5]. The first pathological change in the development of diabetic foot ulcer is vasoconstriction associated with vascular abnormalities, such as thickening of the basement membrane of the capillaries and hyperplasia of their endothelial cells lining with subsequent diminished oxygen tension and hypoxia, as the disease progresses, neuronal dysfunction occurs[1]. Microvascular changes occurs early in diabetes, parallel to the progression of neuronal ischemia which is a characteristic feature in diabetic neuropathy thus both vascular and neural abnormalities determine the severity of structural, functional, and clinical dysfunction [6].

Author α: M.B.Ch.B.; MSc. Ph.D. e-mail: munahzuhair@yahoo.com
Author σ: M.B.Ch.B.; Ph.D. /UK. Department of Anatomy, College of Medicine, University of Mosul, Mosul, Iraq.
Author ρ: F.I.B.M.S Department of Surgery, Al- Jumhuri Teaching Hospital, Mosul.

© 2016 Global Journals Inc. (US)

Diabetic neuropathy is the major problem in diabetes mellitus, which may be prevented by the control blood glucose level and maintenance of normoglycaemia[7]. The term "diabetic foot" involves multiple changes on the level of small and large blood vessels, nerves, bone and soft tissues besides abnormalities of the microcirculation which results in capillary insufficiency, all lead to alterations in the foot biomechanics which promotes tissue destructions and severe infections even sometimes, resulting in amputations[8]. The microangiopathy in diabetes can affect different organs to a different degree, like diabetic nephropathy or retinopathy) (9). Recently it is well-established that there is a close connection between the abnormalities of the microcirculation and the diabetic neuropathy [10] proving the fact that microvascular changes are closely linked to the diminished nervous conduction and the potential of muscular action[11]. The abnormal neural function contributes to the development of microangiopathy in diabetic foot ulcer manifested as thickening of the basal membrane of the capillaries and proliferation of the endothelial cells lining of both arteries and arterioles, resulting in ischemic changes and ulceration[12]. A great improvement in the field of management of diabetic foot has been made by increasing range of the antibiotic therapy and by exploring invasive and noninvasive angiographic techniques [13].

Our present study aims to investigate the histological and vascular changes accompanied to the diabetic foot ulceration and to evaluate the severity and pattern of progress of the condition in relation to the type of diabetes and the control of blood glucose level.

II. Patients, Materials and Methods

In this study, 30 patients were classified into 3 groups 10 patients in each. Group I is the control group have no history of diabetes, group II suffering from diabetes mellitus type I, and Group III patients with diabetes type II. The specimens of skin fragments were collected from the area surrounding the diabetic foot ulcer from diabetic patients who were admitted for surgical management. Detailed history, thorough physical examination and biochemical testing including serum glucose, renal function and liver function tests and x-ray of the affected foot were carried out, the history includes their age, sex, occupation, duration of diabetes, drug history and family history to exclude genetic predisposition of the condition. All the diabetic patients were affected by peripheral neuropathy and showed various degrees of skin lesions in the foot. Specimens of skin tissue were obtained from Al-Jumhuri Teaching Hospital and the histological analysis was performed in the Department of Anatomy, College of Medicine, University of Mosul from November 2015 to June 2016.

Following the debridement of the foot ulceration and removal of the skin tissue fragments from inside and around the ulcer, the specimens were put in a fixative solution (10% neutral formalin) for 24 hour then each specimen was cut into 1 cm thick slices and dehydrated in graded alcohol solutions (70% alcohol for overnight, two changes in 90% alcohol one hour for each and two changes in 100% alcohol for two hours) then the specimens were immersed in xylene using three changes with one-hour interval for each. Complete removal of the clearing solution was made by immersing the tissue specimens into three successive paraffin bathes in oven, one hour for each. Finally paraffin blocks were prepared by embedding the tissue specimens using paraffin wax (melting point is 55-60Co) and these paraffin blocks were now ready for sections using Reichert Rotary Microtome, serial paraffin sections of 4 micrometers in thickness were cut from each block, the sections were collected and mounted on glass slides then the sections were stained with Haematoxylin and Eosin for histological analysis.

III. Results and Observations

This study was performed on 30 persons, 20 of them were diabetic and 10 of them were not, 8 (40%) male and 12 (60 %) female, 14(70%) smoker and 6(30%) were not, 16 (80%) hypertensive and 4(20%) were not, and ١٨(90%) suffering from kidney disease and ٢(10%) were not.

© 2016 Global Journals Inc. (US)

Table 1 : Statistical significance of the demographic variables in patients with diabetes mellitus (N=20)

Variables		Number (N=30)	Percentage %(N=30)	P-values
Sex	Female	١٢	60%	0.01(S)
	Male	٨	40%	0.1(NS)
Smoking	Yes	14	70%	0.02(S)
	No	6	30%	0.2(NS)
Hypertension	Yes	16	80%	0.02(S)
	No	4	20%	0.2(NS)
Renal insufficiency	Yes	18	90%	0.03(S)
	No	2	10%	0.3(NS)

S=Significant (P≤0.05); NS=Non-significant (P>0.05)

The table showed that female affected with diabetes more than male, and statistical significant increase in the incidence of diabetes in smokers (70%) and in those suffering from hypertension (80%) and renal insufficiency (90%) thus P≤0.05.

The histopathological examination of skin fragments from tissue around revealed specific microscopic changes which reflect the pathogenic background of the lesions of diabetic foot.

IV. Histological Findings

a) In the control group

- Skin tissue from the control group showed normal epidermis as stratified squamous epitheliumand dermis which a connective tissue layer (Figure 1).

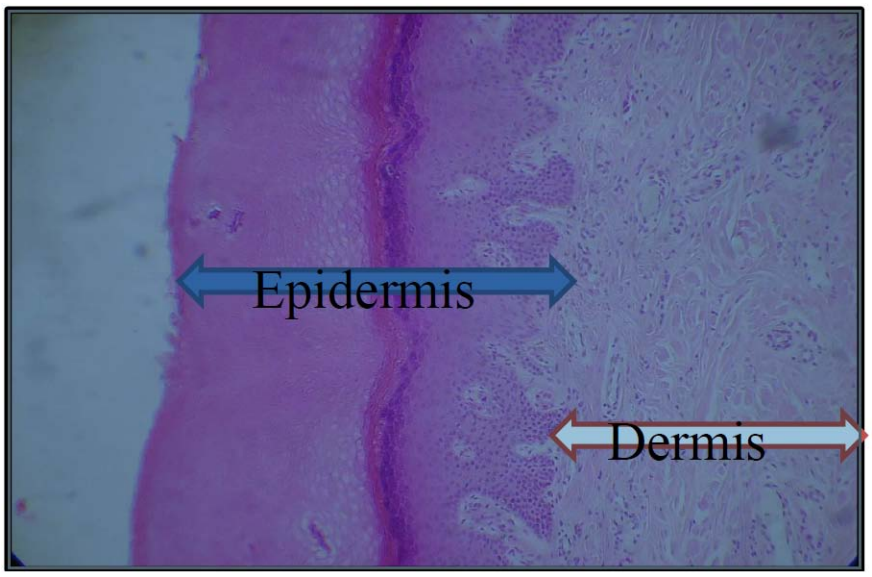

Figure 1 : Photomicrograph of skin tissue from control groupshowed normal epidermis and dermis(H&E X100).

© 2016 Global Journals Inc. (US)

b) Ininsulin dependent diabetes (IDD)

- Mild hyperkeratosis in the epidermis (increase thickness of the keratin layer of the epidermis) with regular acanthosis due to hyperplasia of the stratum spinosum in addition to dense chronic inflammatory cells infiltration mainly lymphocytes and eosinophils and at some time associated with polymorphonuclear neutrophils (Figure 2).

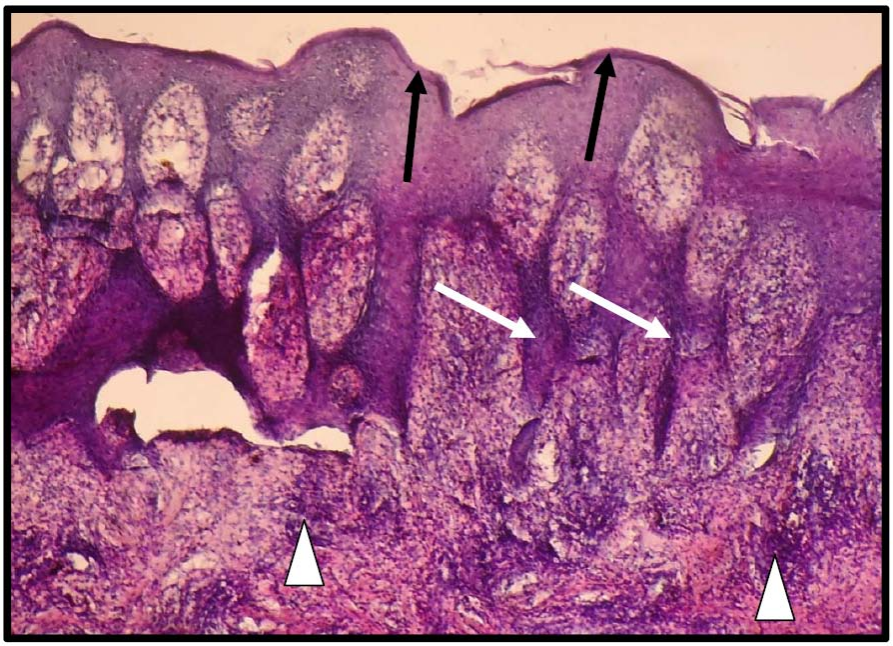

Figure 2 : Photomicrograph of skin tissue from Group II showedmild hyperkeratosis in the epidermis(black arrows) with regular acanthosis(white arrows) anddense chronic inflammatory cells infiltration (arrow heads)(H&E X150).

- Disorganization and degenerative changes of the sweat gland which are surrounded by prominent deposition of lymphocytes (Figure 3).

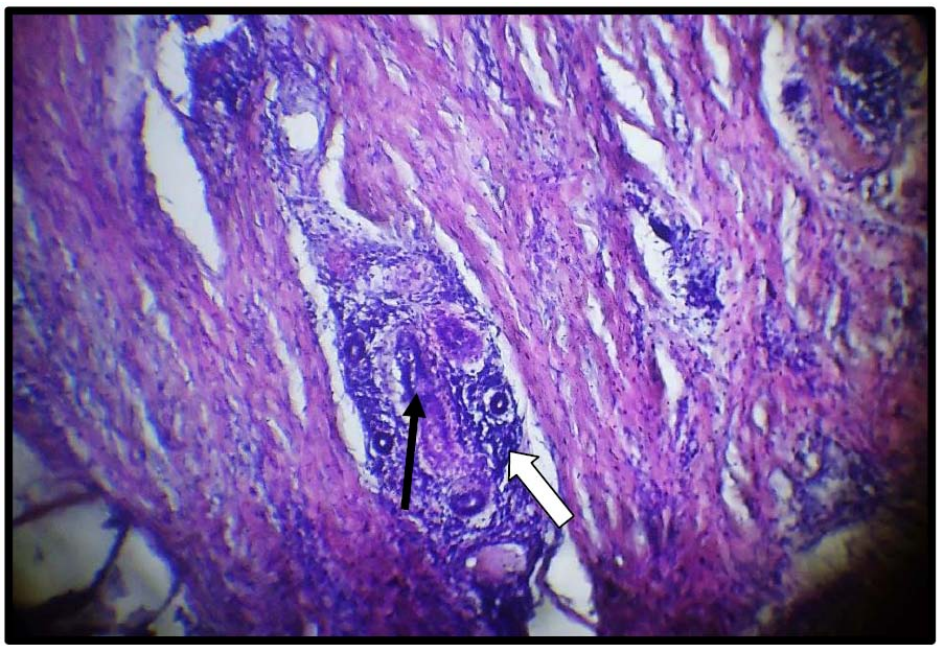

Figure 3 : Photomicrograph of skin tissue from Group II showeddegenerative changes of the sweat gland (black arrow) surrounded by prominent deposition of lymphocytes (white arrow)(H&E X150).

- Congestion of the blood vessels in the dermis with obvious perivascular lymphocytic infiltration arranged in concentric layers "muffs" around them, sometimes penetrating to the media layer (Figure 4).

© 2016 Global Journals Inc. (US)

Figure 4 : Photomicrograph of skin tissue from Group II showed congestion of the blood vessels in the dermis(black arrow) with obvious perivascular lymphocytic infiltration (white arrows)(H&E X100).

- Disturbance of the histological architecture with focal areas of necrosis involving the destruction of the vascular structures **(Figure 5).** Focal melanin pigmentation in the dermis **(Figure 6).**

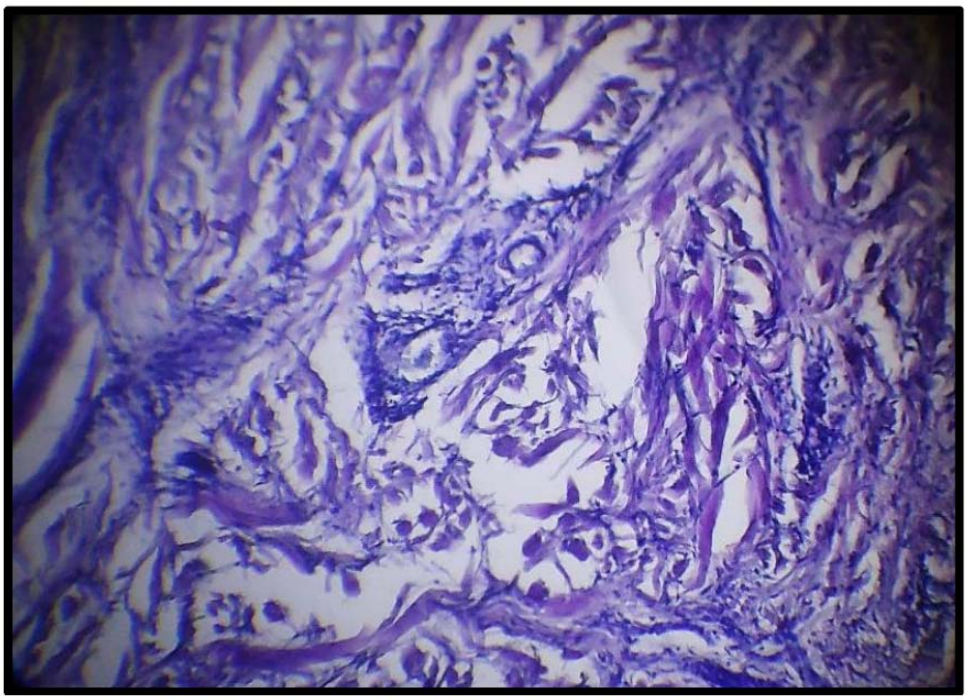

Figure 5 : Photomicrograph of skin tissue from Group II showeddisturbance of the histological architecture with focal areas of necrosis (black arrow)(H&E X100).

© 2016 Global Journals Inc. (US)

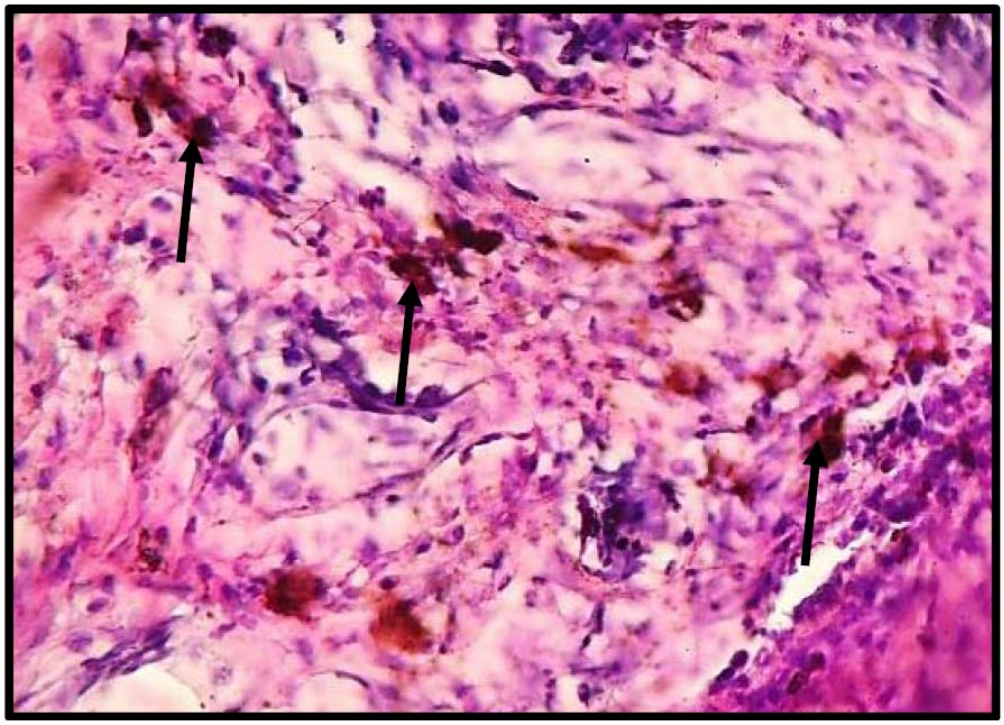

Figure 6 : Photomicrograph of skin tissue from Group II showedfocal melanin pigmentation in the dermis(black arrows)(H&E X150).

- The large arterioles and arteries of muscular type showed swollen endothelial cells lining, excessive proliferation of the subendothelial connective tissue layer and **fibrous tissuedeposition** at the level of media **(Figure 7).**

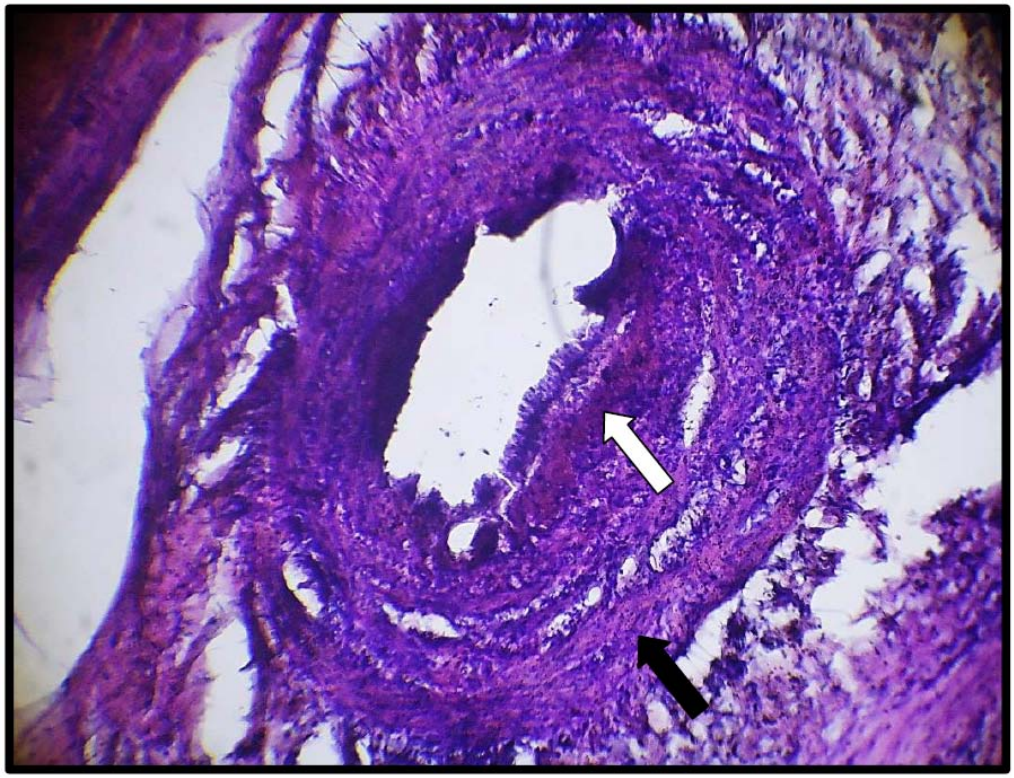

Figure 7 : Photomicrograph of skin tissue from Group II showed the artery with excessive proliferation of the subendothelial connective tissue layer (white arrow)and fibrous tissue deposition in the media (black arrow) (H&E X150).

- Heavy collagen fiber deposition around the blood vessels on using Orcien-Van Gieson stain **(Figure 8).**

© 2016 Global Journals Inc. (US)

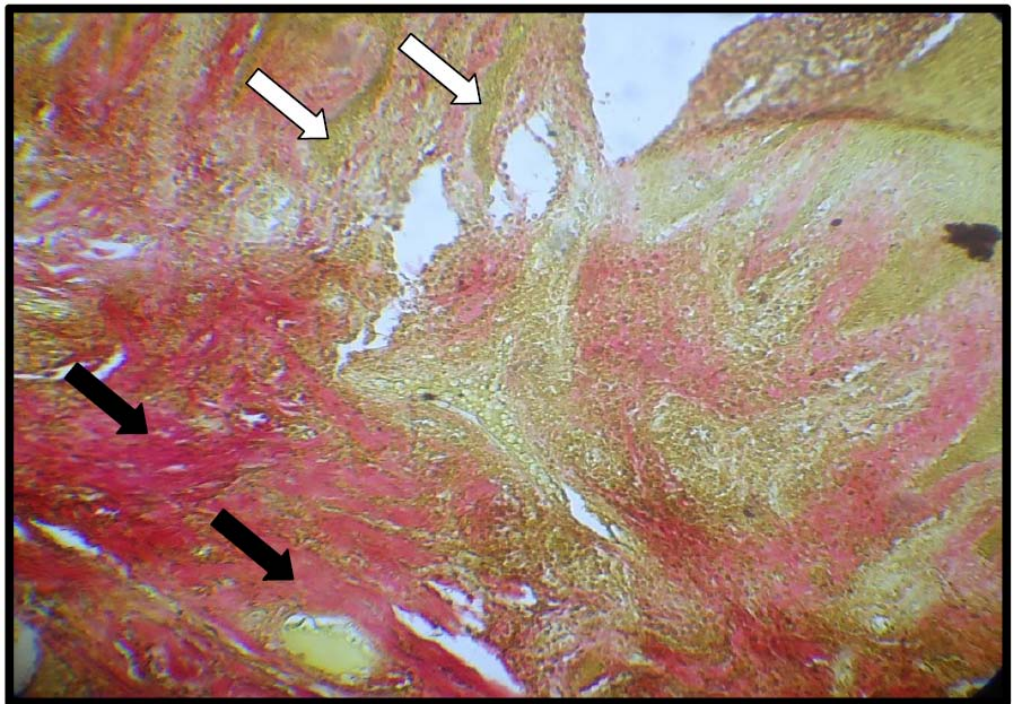

Figure 8 : Photomicrograph of skin tissue from Group Ishowedhyperacanthosis of epidermis (white arrows) with heavy collagen fiber deposition around the blood vessels (black arrows)(Orcien-Van Gieson X400).

c) *In Non-insulin dependent diabetes (NIDD)*

- Marked hyperkeratosis more than insulin dependent diabetes appeared as thickened keratin covering the epidermis with obvious sever acanthosis **(Figure 9).**

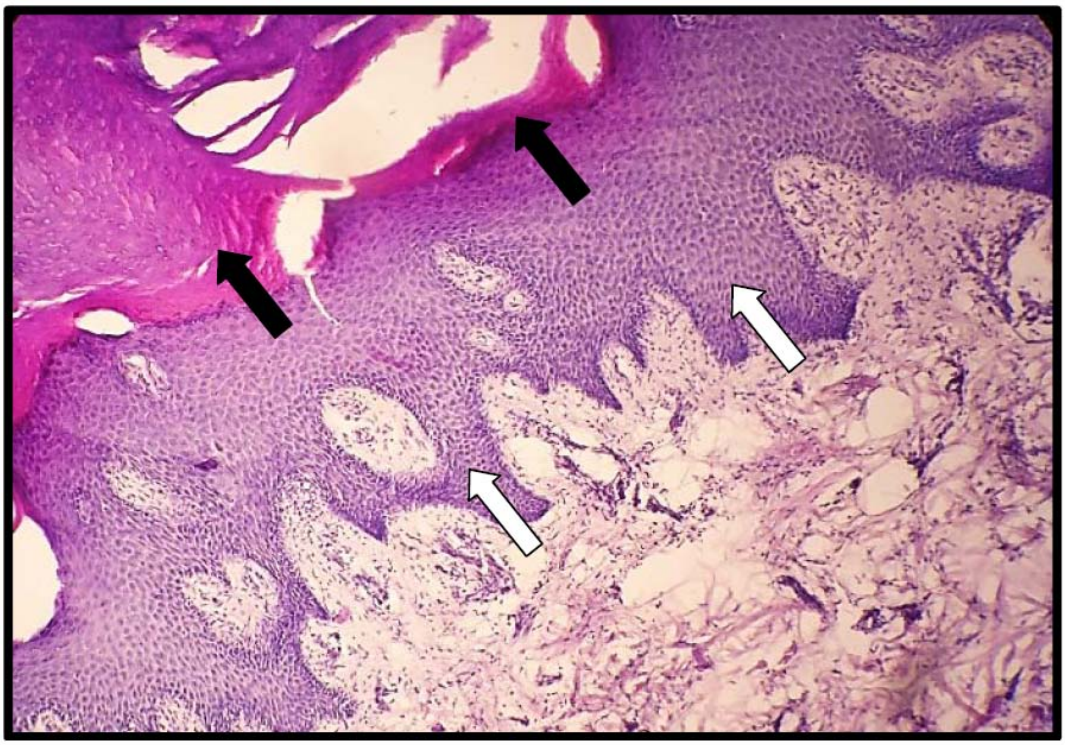

Figure 9 : Photomicrograph of skin tissue from Group III showedmarked hyperkeratosis as thickened keratin covering the epidermis (black arrow)with sever acanthosis (white arrows)(H&E X100).

- Mild inflammatory cells deposition including lymphocytes and plasma cells **(Figure 10).**

© 2016 Global Journals Inc. (US)

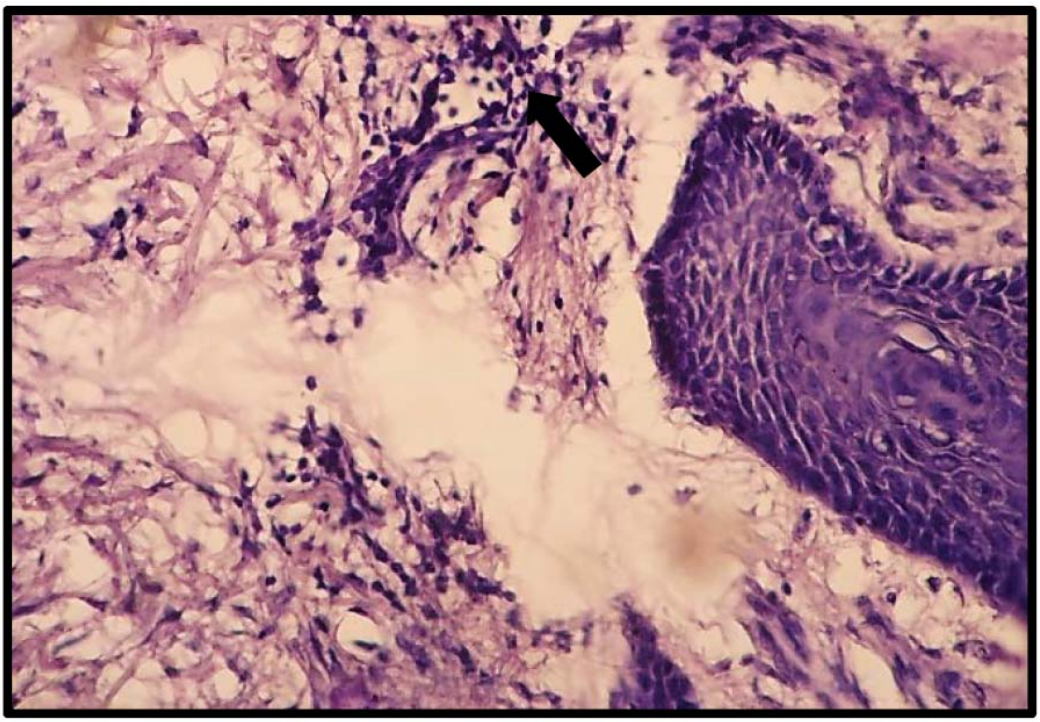

Figure 10 : Photomicrograph of skin tissue from Group III showedmild inflammatory cells deposition in thedermis (black arrow)(H&E X150).

- Dilated acini of the sweat glands with degenerative changes of their lining epithelium and dilatation of their ducts (Figure 11).

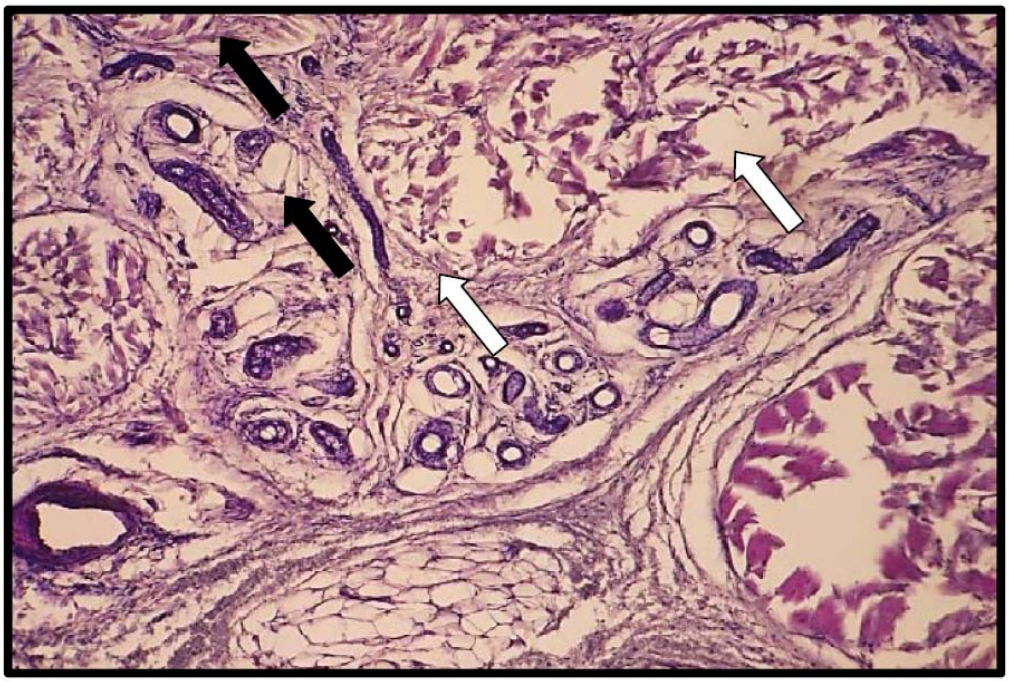

Figure 11 : Photomicrograph of skin tissue from Group III showeddilated acini of the sweat glands with degenerative changes of their lining epithelium (black arrows) and dilatation of their ducts (white arrow)(H&E X150).

- The peripheral nerves showed an obvious vacuolar degeneration of Schwann cells that eventually have resulted in the disappearance of the nerve fibers (Figure 12).

© 2016 Global Journals Inc. (US)

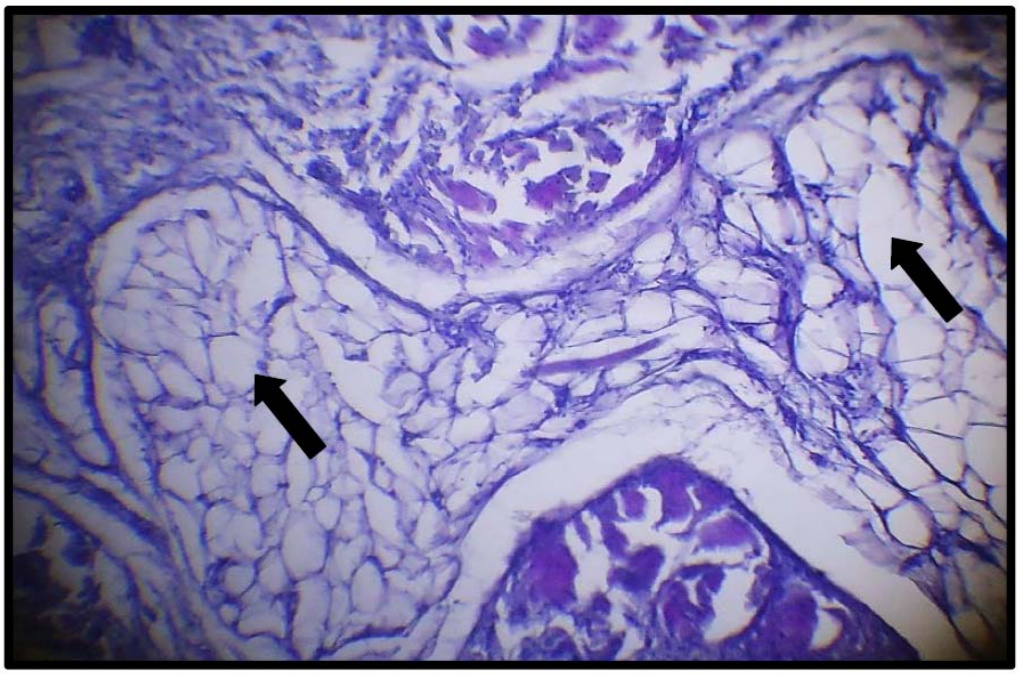

Figure 12 : Photomicrograph of skin tissue from Group III showedvacuolar degeneration of Schwann cells of the peripheral nerve(black arrows)(H&E X400).

V. Discussion

The duration of diabetes, hypertension, smoking, and raised serum cholesterol are important risk factors for the development of diabetic foot ulcers in patients with diabetes. However, various pathogenic mechanisms of vascular and haemodynamic dysfunction have been proposed[14]. Platelet dysfunction, immunological mechanisms, presence of adhesion molecules have been described in relation to diabetic foot[1].

Aguiar et.al, 2007[15] stated that microcirculation is involved in the pathogenesis of diabetic foot which is sometime termed as "small vessel disease".

Endothelial dysfunction precedes the appearance of the microvascular lesions and it is proven by vasoconstriction, marked increase in the microvascular blood flow and vascular permeability and alterations of anti-thrombotic properties of the endothelium[16].

In this study, hyperkeratosis with regular acanthosis in the epidermis and dense chronic inflammatory cells infiltration might be due to release of proinflammatory cytokines like prostaglandins, leukotrienes, and interleukins which cause inflammatory response. This finding previously reported by[17]. Furthermore, vascular congestion in the dermis could be due to the release of vasodilator substances then the stagnant blood in the dilated vessels will cause tissue hypoxia followed by degenerative changes of the sweat gland and focal areas of necrosis. The finding agrees with that observed by[١٨]. deposition of collagen fibers occurs due to chronic inflammatory reaction, thus more fibroblasts might reach the area leading to more collagen fibers deposition[١٩]. Moreover, it has been suggested that alveolar macrophages may release fibroblast chemotactic factors leading to more fibroblast proliferation and **fibrous tissuedeposition** at the level of media[٢٠].

The focal necrotic areas could be provoked by mitochondrial changes mediated by oxidative stress. Ibrahim, (2013)[21] stated that oxidative mitochondrial swelling may lead to rupture of the outer mitochondrial membrane and release of cytochrome C to activate the proapoptoticBax protein which triggers cellular apoptosis followed by necrosis depending on the level of ATP.

Abnormalities in the vascular structure and function are caused by loss of the sympathetic tone, especially on the level of capillaries and small and medium arterioles leading to local ischemia, increase of the arteriolar resistance and consequently a decrease of the blood flow and nutritive circulation of the tissues [22]. The alterations of the microcirculation can also explain the late healing diabetic foot ulcer and even a raised suspicion of infections [23]. The frequency and severity of wound infection may be related to high glucose levels and the contribution of occlusive microvascular disease [24]. During the progression of the diabetic foot ulceration, the nervous involvement is sustained by the morphological changes on the level of the peripheral nerve [25].

Our present study makes a difference by identifying the vascular and nervous changes on the level of cutaneous areas, along with the presence of the

© 2016 Global Journals Inc. (US)

inflammatory infiltrate on the dermis and structural modifications of the sweat glands.

VI. Conclusions

- The identification of vascular and nervous morphological structures in the complicated diabetic foot allows the extension of the knowledge related to the pathological background of this condition.
- With the progression of diabetes mellitus, the vascular lesions, which appeared on the microcirculation level are aggravating consequently involving arterioles and arteries of muscular type and are being accompanied by nervous lesions shown through morphological changes of the peripheral nerves and these changes were accompanied with lesions involving the epidermis, dermis, and muscles.

VII. Recommendations

- The microvascular changes in relation to the severity and progress of the diabetic foot ulceration and the role played by the vascular mediators such as serotonin, 5-hydroxt tryptamine in diabetes require further studies and recordings using both light and electron microscopy.
- Angiographic studies of medium and small arteries to investigate occlusive changes in diabetic foot ulceration were recommended.
- Epidemiological studies to evaluate the influence of diabetes and its outcome on the quality of life particularly in patients with chronic ischemia.

References Références Referencias

1. Rajbhandari SM, Piya MK, A brief review on the pathogenesis of human diabetic neuropathy: Observations and Postulations.Int J Diabetes & Metabolism 2005; 13: 135-140
2. Mekkes JR, Loots M, Bos JD Causes, investigation and treatment of leg ulceration. British Journal of Dermatology 2003; 148: 388-40.
3. Kempler P, Neuropathies. Pathomechanism, clinical presentation, diagnosis, therapy. Springer Scientific Journal 2002; 10 (8):142–149.
4. Reiber GE, Lipsky BA, Gibbons GW. The burden of diabetic footulcers. American Journal of Surgery 2000; 176(2): 5–10.
5. Shaw JE, Boulton AJ. The pathogenesis of diabetic foot problems: An overview. Diabetes 2000; 46 (2): 58-61.
6. Dinh TL, Veves A. A review of the mechanisms implicated in the pathogenesis of the diabetic foot, International Journal of Lower Extremity Wounds 2005; 4(3): 154–159.
7. Schaper NC, Nabuurs MH. The diabetic foot: pathogenesis and clinical evaluation, Journal on Vascular Medicine 2002; 2(2): 221–228.
8. Defronzo RA, ReasnerC. The Diabetes Control and Complications Trial Study: implications for the diabetic foot Journal of Foot Ankle Surgery 2000; 33 (6): 551–556.
9. Hile C, Vevas A. Diabetic neuropathy and microcirculation, Current Diabetic Reproduction 2003; 3(6):446–451.
10. Flynn MD, Tooke JE. Diabetic neuropathy and the microcirculation, Diabetic Medicine 2001; 12 (4): 298–301.
11. Ogawa K, Sasaki H, Yamasaki H, Okamoto K, MatunoS, Shono T, Arimoto K, Furuta H, Nishi M, NakaoT, Nanjo K. Peripheral nerve functions may deteriorate parallel to the progression of microangiopathy in diabetic patients, Nutritional Metabolic Cardiovascular Disease 2006; 16(5): 313–321.
12. Malik RA, Newrick PG, Sharma AK, Jennings A, Ahsee K, Mayhew TM, Jakubowski J, Boulton AJ, Ward JD. Microangiopathy in human diabetic neuropathy :relationship between capillary abnormalities and the severity of neuropathy, Diabetologia 2000; 32(2): 92–102.
13. Flynn MD, Tooke JE. Aetiology of diabetic foot ulceration: A role for the microcirculation, Diabetic Medicine 2001; 9(4):320– 329.
14. Dinh T, Tecilazich F, Kadomas A. Mechanisms involved in the development and healing of diabetic foot ulcer, Am Diabetes Association 2012; 16 (11): 2937-2941.
15. Aguiar LG, Villela NR, Bouskela. Microcirculation in diabetes: implications for chronic complications and treatment of the disease, Endocrinological Metabolism 2007; 51(2): 204–211.
16. Esper RJ, Vilarino JO, Machado RA, Paragano A. Endothelial dysfunction in normal and abnormal glucose metabolism, Advanced Cardiology 2008; 45: 17–43.
17. Wang YN, Lee K, LedouxWR, Histomorphological evaluation of diabetic planter soft tissue. Foot Ankle Int. 2014; 32(8): 802-810.
18. Lew E, Nicolosi N, BotekG, Lower extremity amputation risk factor associated with elevated ankle brachial indices and radiographic arterial calcification. J Foot Ankle Surgery 2015; 54: 413-417.
19. Kirsner RS, Vivas AC, Lower extremity ulcer: Diagnosis and Management. British J of Dermatol. 2015; 137: 379-90.
20. Yang MY, Kim GW, Song M, Bullae and sweat gland necrosis with concurrent muscle infarction in patient with long standing type I diabetes. Paper Search.net, Korean Study 2015; 120: 179-85.

© 2016 Global Journals Inc. (US)

21. Ibrahim A Vitamin A downregulating Bcl-2 and TGF-α expression duringcolon cancer in AFB1-induced female rats, J Natural Sciences Research 2013; 3(5): 67-83.
22. Lafontaine J, Harkless LB, Davis CE, Allen MA, Shireman PK. Current concepts in diabetic microvascular dysfunction, J. Am Podiatr Medical Association 2006; 96(3): 245–252.
23. Korzon A, Edmonds M. Role of the microcirculation in diabetic foot ulceration, International Journal of Lower ExtremityWounds 2006; 5(3): 144–148.
24. Edmonds M. Diabetic foot ulcers: practical treatment recommendations, Drugs 2006; 66(7): 913– 929.
25. Chantelau E. Obliterating diabetic microangiopathy of the diabetic foot, Gesamte International Medical Journal 2000; 48(8): 376–380.

© 2016 Global Journals Inc. (US)

This page is intentionally left blank

© 2016 Global Journals Inc. (US)

Global Journal of Medical Research: C
Microbiology and Pathology
Volume 16 Issue 1 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

Bacteriological Profile and Antibiotic Susceptibility Pattern of Blood Culture Isolates from Patients Visiting Tertiary Care Hospital in Kathmandu, Nepal

By Prakash Simkhada, Shiva Raj K. C., Santosh Lamichhane, Surya Subedi & Upendra Thapa Shrestha

Tribhuvan University, Nepal

Abstract- Bacterial blood stream infections can lead to life threatening sepsis that requires rapid antimicrobial treatment otherwise may lead to morbidity and mortality of patients. Blood culture is gold standard technique which provides essential information for the diagnosis and appropriate medication to save life of affected patients. Present study was conducted to determine the bacteriological profile of blood stream infections and their antibiotic susceptibility pattern in patients visiting Janamaitri Hospital, Balaju, Kathmandu, Nepal. A total of 838 blood samples were collected from the clinically suspected cases of bacteremia and septicaemia. Isolates were identified by standard biochemical tests, and antibiotic susceptibility test was performed by using CLSI guidelines. Positive blood culture was obtained in 61/838 (7.28%) where gram negative accounted for 48/61 (78.69%) in which *Salmonella* Paratyphi A was leading organisms and gram positive accounted for 13/61(21.31%) in which *Staphylococcus aureus* was leading organisms.

Keywords: blood stream infections, multi-drug resistant, salmonella paratyphi A, janamaitri hospital.

GJMR-C Classification : NLMC Code: QW 4 , QW 50

BACTERIOLOGICALPROFILEANDANTIBIOTICSUSCEPTIBILITYPATTERNOFBLOODCULTUREISOLATESFROMPATIENTSVISITINGTERTIARYCAREHOSPITALINKATHMANDUNEPAL

Strictly as per the compliance and regulations of:

Open Association of Research Society, USA
Research | Diversity | Ethics

© 2016. Prakash Simkhada, Shiva Raj K. C., Santosh Lamichhane, Surya Subedi & Upendra Thapa Shrestha. This is a research/review paper, distributed under the terms of the Creative Commons Attribution-Noncommercial 3.0 Unported License http://creativecommons.org/ licenses/by-nc/3.0/), permitting all non-commercial use, distribution, and reproduction in any medium, provided the original work is properly cited.

Bacteriological Profile and Antibiotic Susceptibility Pattern of Blood Culture Isolates from Patients Visiting Tertiary Care Hospital in Kathmandu, Nepal

Prakash Simkhada [α], Shiva Raj K. C. [σ], Santosh Lamichhane [ρ], Surya Subedi [ω] & Upendra Thapa Shrestha [¥]

Abstract- Bacterial blood stream infections can lead to life threatening sepsis that requires rapid antimicrobial treatment otherwise may lead to morbidity and mortality of patients. Blood culture is gold standard technique which provides essential information for the diagnosis and appropriate medication to save life of affected patients. Present study was conducted to determine the bacteriological profile of blood stream infections and their antibiotic susceptibility pattern in patients visiting Janamaitri Hospital, Balaju, Kathmandu, Nepal. A total of 838 blood samples were collected from the clinically suspected cases of bacteremia and septicaemia. Isolates were identified by standard biochemical tests, and antibiotic susceptibility test was performed by using CLSI guidelines. Positive blood culture was obtained in 61/838 (7.28%) where gram negative accounted for 48/61 (78.69%) in which *Salmonella* Paratyphi A was leading organisms and gram positive accounted for 13/61(21.31%) in which *Staphylococcus aureus* was leading organisms. The most effective antibiotics for gram negative organisms was imipenem followed by amikacin whereas for gram positive was vancomycin whereas imipenem and amikacin showed wide range of effectiveness for both organisms. All isolates of *Escherichia coli*, *Morgenella morganii*, *Staphylococcus aureus*, coagulase-negative staphylococci, *Streptoccus pyogenes* were multidrug-resistant. *Salmonella* Paratyphi B does not showed resistance to more than one drug and found most sensitive organisms among isolated ones.

Keywords: blood stream infections, multi-drug resistant, salmonella paratyphi A, janamaitri hospital.

I. Introduction

Bacteremia is the presence of viable bacteria in the circulating blood. The detection of bacteria in blood is always abnormal. A minor injury occurring during tooth brushing, tooth extraction, abscesses, infected wound or boils, insertion of intravenous of bladder catheter, surgery and existing infections like lung infection, Urinary tract infection (UTI), gastrointestinal tract (GTI), burns or bedsores or from areas of localized disease as in pneumococcal pneumonia, meningitis, pyelonephritis, osteomyelitis, cholangitis, peritonitis, enterocolitis and puerperal sepsis are the sources of bacteremia and blood culture is required for the detection of it [1]. Bloodstream infection (BSI) is one of the most important causes of morbidity and mortality globally [2]. Detection of bacteremia by rapid and reliable method is by culturing blood. The blood should be collected aseptically before the administration of antibiotics [3]. Septicaemia is a clinical term used to describe severe life-threatening bacteremia in which multiplying bacteria release toxins into the blood stream and trigger the production of cytokines, causing fever, chills, toxicity, tissue anoxia, reduced blood pressure and collapse. Septic shock is usually a complication of septicemia with Gram-negative bacilli, and less frequently, Gram-positive organisms and prompt treatment is essential [4]. Continuous septicemia occurs primarily in patients with intravascular infections like endocarditis, septic thrombophlebitis, infections associated with intravascular catheter, septic shock whereas intermittent septicemia occurs in patients with localized infections like lung, urinary tract, soft tissues infections [5].

Bloodstream infections are potentially life-threatening and require rapid identification and antibiotic susceptibility testing of the causative pathogen. Both Gram positive and Gram negative bacteria causes bacteremia and septicemia. Gram negative septicemia, also known as endotoxic shock, which is more severe than Gram positive septicemia [6].

If the infection is caused by multidrug resistant (MDR) bacteria morbidity and mortality will increase which leads to great economic loss encompassing use of more expensive antibiotics to treat infection as well as threat of resistance to them. The infections caused by MDR organisms are more likely to prolong the hospital stay, increase the risk of death and require treatment with more expensive antibiotics [7].

In almost all cases, antimicrobial therapy is initiated empirically before the results of blood culture are available by keeping in mind that high mortality and

Author α ρ ¥: Department of Microbiology, Kantipur College of Medical Science, Kathmandu, Nepal.
e-mails: prakashsimkhada1986@gmail.com, rajeshworlamichhane@gmail.com
Author σ: Janamaitri Hospital Balaju, Kathmandu, Nepal.
Author ω: Tri-Chandra Multiple College, Ghantaghar, Kathmandu, Nepal. e-mails: suryasubedi42@gmail.com, subedi_010@yahoo.com

© 2016 Global Journals Inc. (US)

morbidity are associated with septicemia and right choice of empiric therapy is of importance [6]. The increasing frequency of antimicrobial resistance among microbial pathogens causing nosocomial and community acquired infections is making numerous classes of antimicrobial agents less effective resulting in emergence of antimicrobial resistance [7, 22]. For the treatment, the isolation of bacterium from blood is valuable, but there is also urgent need of antimicrobial therapy, so sample is taken and treatment is started and after blood culture result, patient is treated as redirected by in vitro antibiotic sensitivity test [8, 9]. Therefore, we conducted this study to determine the common bacterial agents associated with bacteremia and their antimicrobial susceptibility patterns in febrile patients visiting in Janamaitri Hospital, Balaju, Kathmandu, Nepal.

II. Materials and Methods

The study was conducted in Microbiology Laboratory of Janamaitri Hospital, Kathmandu, Nepal from March 2014 to April 2015. Written informed consents were obtained from patients prior to their inclusion in the study. A total of 838 blood samples were processed during the research period. Five ml blood sample was collected from each adult, 2-5ml from each child and 0.5-2ml from infant's aseptically using 70% alcohol and 2% tincture of iodine and inoculated immediately into 50ml Brain Heart Infusion (BHI) Broth with 0.025% of sodium polyanethol sulphonate as anticoagulant. In pediatrics cases, 1-2ml of blood was inoculated in 5-10ml of BHI broth. Negative result was followed by examining the broth daily for the sign of bacterial growth (turbidity, haemolysis, clot formation) and by doing final subculture at the end of seventh day. Bottles that showed sign of growth were further processed by Gram stain, followed by subculture on Blood agar, Mac Conkey agar, Manitol salt agar and examined after 18-24 hrs of incubation. Bacterial isolates were identified by colony morphology, Gram staining, catalase test, coagulase test, oxidase test, methyl red/voges-proskauer test (MR-VP), Triple sugar iron agar test, citrate utilization test, Urease test and Sulfur Indole Motility (SIM) test using standard procedure for bacterial identification [10].

a) *Antibiotic susceptibility test*

Antimicrobial susceptibility testing was performed by using Kirby Bauer disc diffusion method following guidelines of Clinical and Laboratory Standard Institute 2012 [11]. The inoculums used for susceptibility testing was prepared in nutrient broth by touching 5/6 colony and matched to 0.5 McFarland standard (1.5 X 10^8 CFU/ml). Within 15 minutes, a sterile cotton swab was dipped into the inoculums suspension and pressed inside the wall of tube above the fluid level and inoculated at 60°C over the dried surface of Muller-Hilton agar (MHA) plate. After 3-5 minutes of inoculation, the antibiotic discs were applied and gently pressed down to ensure complete contact with agar. Organisms which showed resistance to at least one antibiotic among three or more antimicrobial categories were considered as multidrug resistant (MDR) bacteria [12, 13, 14].

b) *Quality control*

Reference strains *E. coli* (ATCC 25922) and *S. aureus* (ATCC 25923) were used as a control reference strains for identifications and drug susceptibility testing [14, 15].

c) *Data analysis*

The data obtained from the research were analyzed by using statistical tools in SPSS-21 version. The chi-square test was used for statistical analysis of data. A 'P' value less than 0.05 was considered as statistically significant.

III. Results

A total of 838 clinical blood samples were collected from the patients attending Janamaitri Hospital, Kathmandu to study the prevalence of bacteraemia and septicemia from 9th August, 2014 to 8th November, 2015.

a) *Ward wise distribution of positive samples*

There were all together 838 blood samples, out of which, 61(7.28%) samples showed growth and rest 777 (92.72%) showed no growth. Again higher percentage of growth was obtained from OPD (Outpatient Department) followed by emergency and wards (Table 1).

Table 1 : Ward wise distribution of positive samples

Types of patients	Growth		Total samples	
	Number	%	Number	%
OPD	41	67.21	476	56.80
Emergency	14	22.95	261	31.15
Wards	6	9.84	101	12.05
Total	61	100	838	100

© 2016 Global Journals Inc. (US)

b) Pattern of bacteria isolated from blood culture

Out of 61 bacterial isolates, 48 (78.69%) were Gram negative and 13 (21.31%) were Gram positive bacteria. Among Gram negative bacterial isolates *Salmonella* Paratyphi A 26/48 (54.17%) was found to be most predominant among all bacterial isolates followed by *Salmonella* Typhi (33.33%), *Escherichia coli* (6.25%), *Salmonella* Paratyphi B (4.17%), *Morganella morganii* (2.08%). In Gram positive isolates *Staphylococcus aureus* 5/13(38.46%) was most predominant followed by coagulase-negative staphylococci (CNS) 4/13 (30.77%), *Streptococcus pyogenes* 2/13 (15.38%) and *Enterococci* 2/13 (15.38%) (Table2).

Table 2 : Pattern of bacteria isolated from blood culture gender wise

Bacteria	Number	Percentage	Male		Female	
			Number	%	Number	%
Gram negative						
Escherichia coli	3	6.25	1	2.08	2	4.17
Morgenella morganii	1	2.08	1	2.08	0	0
Salmonella Paratyphi A	26	54.17	18	37.50	8	16.67
Salmonella Paratyphi B	2	4.17	2	4.17	0	0
Salmonella Typhi	16	33.33	9	18.75	7	14.58
Total	48	78.69	31	64.58	17	35.42
Gram positive						
Staphylococcus aureus	5	38.46	3	23.08	2	15.38
CNS	4	30.77	2	15.38	2	15.38
S. pyogenes	2	15.38	2	15.38	0	0
Enterococci	2	15.38	0	0	2	15.38
Total	13	21.31	7	54.85	6	46.15

c) Age wise distribution of the isolates

Out of 61 bacterial isolates, highest number of bacteria was isolated from age group 16-30 years 33(54.10%) followed by the age group 31-45 years 13(21.31%) and age group 76-90 years was found to be least affected 1(1.64%). Age group 16-30 years was most affected by wide range of bacteria (Table3).

Table 3 : Age wise and Ward wise distribution of the isolates

Bacteria	Age group (year)						OPD		Emergency		Wards	
	1-15	16-30	31-45	46-60	61-75	76-90	Number	(%)	Number	(%)	Number	(%)
Gram Negative Bacteria												
Escherichia coli	0	3	0	0	0	0	1	2.08	2	4.17	0	0
Morgenella morganii	0	1	0	0	0	0	0	0	0	0	1	2.08
Salmonella Paratyphi A	0	16	6	3	1	0	19	39.58	6	12.50	1	2.08
Salmonella Paratyphi B	1	1	0	0	0	0	1	2.08	1	2.08	0	0
Salmonella Typhi	2	7	4	2	0	1	12	25.00	3	6.25	1	2.08
Total	3	28	10	5	1	1	33	68.75	12	25.00	3	6.25
Gram Positive Negative												
Staphylococcus aureus	1	2	1	0	1		3	23.08	1	7.69	1	7.69
CNS	0	1	2	0	1	0	2	15.38	1	7.69	1	7.69
Streptococcus pyogenes	0	1	0	1	0	0	1	7.69	0	0	1	7.69
Enterococci	1	1	0	0	0	0	2	15.38	0	0	0	0
Total	2	5	3	1	2	0	8	61.54	2	15.38	3	23.08

d) Seasonal variation on the prevalence of bacterial growth

Out of 48 Gram negative organisms isolated, 20(41.67%) showed growth in summer season and followed by autumn season 20 (41.67%). Growth in winter was 4/48 (8.33) and in spring was 4/48 (8.33%). Out of 13 Gram positive bacterial isolates, 6/13(46.15%) showed growth in summer and followed by spring season 4/13(30.77%). Growth in winter was 2/13(15.38%) and in autumn was 1/13(7.69%). Sample showed highest growth in summer and autumn season (Table 4).

© 2016 Global Journals Inc. (US)

Table 4 : Seasonal variation on the prevalence of bacterial growth

	Season			
	Summer	Autumn	Winter	Spring
Gram Negative Bacteria				
Escherichia coli	1	0	1	1
Morgenella morganii	0	0	0	1
Salmonella Paratyphi A	9	14	2	1
Salmonella Paratyphi B	0	1	1	0
Salmonella Typhi	10	5	0	1
Total	20 (41.67%)	20(41.67%)	4 (8.33%)	4 (8.33%)
Gram Positive Bacteria				
Staphylococcus aureus	3	1	0	1
CNS	1	0	1	2
S. pyogenes	1	0	0	1
Enterococci	1	0	1	0
Total	6 (46.15%)	1 (7.69%)	2 (15.38%)	4 (30.77)

e) Antibiotic resistance pattern of Gram negative bacteria

Out of 838 blood samples, 48 different Gram negative bacteria were isolated. Among the isolates, *Salmonella* Paratyphi A was most predominant followed by *Salmonella* Typhi. Five different bacteria were isolated during study period. Antibiotic susceptibility was performed on them in which imipenem was found most effective followed by amikacin (Table 5).

Table 5 : Antibiotic susceptibility pattern of Gram-negative isolates

Gram Negative Bacteria	Antibiotic susceptibility pattern (%) / number											
	AMX	COT	CN	OF	CIP	C	CFM	NA	CTX	GEN	AK	IPM
Escherichia coli (3)	(33.3) 1	(66.67) 2	(33.33) 1	(66.66) 2	(33.33) 1	(33.3) 1	(33.3) 1	-	(100) 3	(100) 3	(100) 3	(100) 3
Morgenella morganii (1)	-	-	-	-	-	100 1	100 1	-	100 1	100 1	100 1	(100) 1
Salmonella Paratyphi A (26)	(96.15) 25	(50) 13	(88.46) 23	(73.08) 19	(73.08) 19	(84.62) 22	(100) 26	-	(100) 26	(96.15) 25	(96.15) 25	(100) 26
Salmonella Paratyphi B (2)	(100) 2	(100) 2	(100) 2	(100) 2	(100) 2	(100) 2	(100) 2	-	(100) 2	(100) 2	(100) 2	(100) 2
Salmonella Typhi (16)	(87.50) 14	(62.50) 10	(81.25) 13	(75.00) 12	(75.00) 12	(75.00) 12	(81.25) 13	-	(93.75) 15	(87.50) 14	(68.75) 11	(100) 16

AMX=Amoxycillin, COT=Cotrimoxazole, CN=Cefalexin, OF=Ofloxacin, CIP=Ciprofloxacin, C=Chloramphenicol, CFM=Cefixime, NA=Nalidixic acid, CTX=Cefotaxime, GEN=Gentamicin, AK=Amikacin, IPM=Imipenem

f) Antibiotic resistance pattern of Gram positive bacteria

Out of 838 blood samples, only 13 samples showed growth of Gram positive bacteria. Isolated bacteria were subjected to antibiotic susceptibility test (Table 6).

Table 6 : Antibiotic susceptibility pattern of Gram-positive isolates

Gram positive bacteria	Antibiotic susceptibility pattern (%) / number											
	P	AMP	C	CIP	GEN	MET	E	VAN	PIP	IPM	AK	CLO
Staphylococcus aureus (5)	0	(20) 1	(40) 2	(40) 2	0	(60) 3	(60) 3	(100) 5	(100) 5	(100) 5	(80) 4	(20) 1
CNS (4)	0	(50) 2	(25) 1	(50) 2	(75) 3	(50) 2	(75) 3	(100) 4	0	(50) 2	(75) 3	(50) 2
Streptococcus pyogenes (2)	0	(50) 1	(50) 1	0	(50) 1	(100) 2	(100) 2	(100) 2	(50) 1	(100) 2	(100) 2	(50) 1
Enterococci (2)	0	0	(50) 1	0	(50) 1	(100) 2	(100) 2	(100) 2	0	(100) 2	(100) 2	0

P= Penicillin, AMP= Ampicillin, C=Chloramphenicol, CIP= Ciprofloxacin, GEN= Gentamicin, MET=Methicillin, E=Erythromycin, VAN=Vancomycin, PIP=Piperacillin, IPM=Imipenem, AK=Amikacin, CLO=Cloxacillin

© 2016 Global Journals Inc. (US)

g) Multi drug resistance of bacterial isolates

Out of 48 Gram neative isolates 15 strains (31.25%) were resistant to ≥3 antibiotics and were considered as multidrug resistant. It was found that 16 (33.33%) isolates were resistant to 1 antibiotic and 17(35.42%) isolates were resistant to 2 antibiotics. Among the MDR strains, 43.75% (7 out of 16) of *Salmonella* Typhi were found to be MDR. Similarly 15.38% (4 out of 26) of *Salmonella* Paratyphi A were found to be MDR. All the isolates (100%) of *Escherichia coli, Morganella morganii*, and *Staphylococcus aureus* were found to be MDR (Table 7).

Table 7 : MDR pattern of the bloodstream isolates

Organisms	Number	Number of bacteria resistance to			MDR	
Gram Negative Bacteria		1 antibiotic	2 antibiotic	≥3 antibiotics	Number	%
Escherichia coli	3	0	0	3	3	100
Morgenella morganii	1	0	0	1	1	100
Salmonella Paratyphi A	26	9	13	4	4	15.38
Salmonella Paratyphi B	2	2	0	0	0	0
Salmonella Typhi	16	5	4	7	7	43.75
Gram positive						
Staphylococcus aureus	5	0	0	5	5	100
CNS	4	0	0	4	4	100
Streptococcus pyogenes	2	0	0	2	2	100
Enterococci	2	0	1	1	2	50.00

IV. Discussion

Blood culture is a well-established procedure of the standard diagnostic workup for many infectious diseases. In the countries like Bangladesh, where all kinds of drugs including the antibiotics, are sold over the counter, misuse of antibiotics has been found to be responsible for developing pool of resistant bacteria as well as negative results of blood culture [16]. Blood culture is employed for the detection of bacteremia and septicemia in blood. Blood stream infection is one of the main agent causing morbidity and mortality worldwide. Urgent and effective treatment is required to manage blood infections [17].

In the countries like Nepal, the overuse of antibiotics, random use of antibiotics as hit and trial method by clinicians without proper sensitivity test, unawareness of people about emergence of antibiotics resistance, random use of antibiotics without advice of physicians, prolonged intensive care unit (ICU) stay, nursing home residency, severe illness, use of instrumentation or catheterization etc. are the major causes of drug resistance in our country. The less growth percentage may due to previous exposure of patients to used antibiotics that hindered their growth or dominance of organism's growth [18].

In the present study the isolation rate was (61/838) 7.28% which was comparable to those study conducted by Karki et al where 4.2% were culture positive [19]. Gram negative bacteria were common organisms isolated during this study accounting 48/61 (78.69%). Among Gram negative isolates the most common was *Salmonella* Paratyphi A 26/48 (54.17%) followed by *Salmonella* Typhi 16/48 (33.33%). Similar finding was seen from the studies in Kathmandu Model hospital Nepal, where 71% of total isolates from blood were *Salmonella* Typhi and 16% of the total isolates were *Salmonella* Paratyphi A [20]. In this study the isolation rate was highest in age group between 16-30 years 33/61 (54.10%) followed by age group 31-45 years 13/61 (21.31%). Similar study conducted in showed that the isolation rate was highest in age group between 21-40 (28%) followed by 41-60 (24%) [21]. In this study, among Gram positive bacteria the most common isolated bacteria was *Staphylococcus aureus* 5/13 (38.46%) followed by CNS 4/13 (30.77%).

The most effective antibiotic in Gram negative bacteria was imipenem followed by amikacin. The others effective drugs were cefotaxime and gentamycin. Similar study conducted in Nepal in KIST Medical College by Surya et al showed that imipenem and amikacin followed by gentamycin were most effective antibiotics for the treatment of *Escherichia coli* and *Klebsiella pneumoniae* [22]. Those organisms which showed resistance to at least one agent in three or more antimicrobial categories were considered as multidrug resistant (MDR) bacteria [23].

In the present study among Gram negative bacteria, 100% of *E. coli* and *Morgenella morganii* isolates were MDR, followed by *Salmonella* Typhi (43.75%) and *Salmonella* Paratyphi A (15.38%). None of the isolates of *Salmonella* Paratyphi B was found to be MDR. Similar study conducted in Nepal showed that 96.10% of the *E. coli* isolates were MDR whereas 44.8% of the *Salmonella* isolates were MDR in Ghana [22, 24].

In this study, among Gram positive bacteria, *S. aureus*, CNS and *Streptococcus pyogenes* were found 100% MDR whereas 50% of *Enterococci* isolates were MDR. Similar study conducted in Ethiopia showed that 100% of the *S. aureus* and CNS were MDR whereas none of the *S. pyogenes* isolates were MDR [2]. In the present study vancomycin is the antibiotic of choice for the treatment of Gram positive bacterial isolates followed by imipenem and amikacin. Higher rate of infection by *Salmonella* species may be due to

© 2016 Global Journals Inc. (US)

unhygienic practices, contaminated food and drinking water. The prevalence of typhoid was higher in summer and autumn season which may be due to contaminated water and food [25, 26].

In this study, Imipenem and Amikacin were found most effective for Gram negative isolates whereas vancomycin was found most effective for Gram positive bacteria followed by imipenem and amikacin. These three antibiotics should not be used indiscriminately and kept as reserve drug because if resistance is developed then treatment will be complicated.

V. Conclusion

Salmonella Paratyphi A and *Salmonella* Typhi was the major Gram negative organisms causing blood stream infections whereas *Staphylococcus aureus* and CNS was the major Gram positive organisms. The antibiotic resistance pattern varies according to geographical location, country to country and even institute to institute in the same country and continuously changes over time so determination of antibiotic sensitivity pattern in periodic intervals is mandatory for choosing appropriate antibiotics for the treatment. The antibiotic susceptibility pattern of this study suggested that vancomycin is the drug of choice for Gram positive bacteria while imipenem and amikacin for Gram negative bacteria but the later drugs showed wide coverage for Gram positive organisms too.

VI. Acknowledgements

We are indebted to Kantipur College of Medical Science for providing fund for this study. We also wish thank to Janamaitri Hospital for providing laboratory facility for the research.

References Références Referencias

1. Trevino S and Ross D. Bacterermia and Sepsis. In: Mahon CR, Lehman DC, Manuselis G. Textbook of Diagnostic Microbiology. 3rd eds., New Delhi: Elsevier; 2007; pp. 995-1009.
2. Zenebe T, Kannan S, Yilma D and Beyene G. Invasive bacterial pathogens and their antibiotic susceptibility patterns In Jimma University Specialized Hospital, Jimma, Southwest Ethiopia. Ethiopian Journal of Health Science, 2011; 21(1): pp. 1-8.
3. William JH and Max S. Bacteremia, septicemia and endocarditis. In: William JH, Max Sussman. Topley and Wilsons Microbiology and Microbial Infections, Vol 3. 11th eds., London; 1998, pp. 178-187.
4. Cheesbrough M. District Laboratory practice in tropical countries. Cambridge University press, Cambridge, UK, 2nd Edition, Part 2, 2006; 124-130.
5. Murray PR, Rosenthal KS and Pfaller MA. Medical Microbiology seventh edition Saunders, an imprint of Elsevier Inc. 2013 pp. 157.
6. A Garg, S Anupurba, and J Garg. "Bacteriological profile and antimicrobial resistance of blood culture isolates from a university hospital," Journal of Indian Academy of Clinical Medicine, 2007; 8 (2): 139–143.
7. Subedi S, Chaudhary M and Shrestha B. High MDR and ESBL producing *Escherichia coli* and *Klebsiella pneumoniae* from urine, pus and sputum. British Journal of Medicine & Medical Research, 2016; 13 (10): 1-10.
8. Collee JG, Duguid JP, Fraser AG, Marmion BP and Simmons A. Laboratory strategies in the diagnosis of infective syndromes. In: Collee JG, Duguid JP, Fraser AG, Marmion BP, Simmons A, editors. Mackie and MacCartney Practical Medical Microbiology. 14th eds., New Delhi, India: Elsevier; 2006, pp. 55-57.
9. Gohel K, Jojera A, Soni S, Gang S, Sabnis R and Desai M. Bacteriological profile and drug resistance patterns of blood culture isolates in a tertiary care nephrourology teaching institute. BioMed Research International, 2014; pp. 1-5.
10. Collee, JG Mackie and Mccartney practical medical microbiology (English), 14th edn, Churchill Livingstone, London 1996; pp. 265-463.
11. Clinical Laboratory Standard Institute Performance Standards for Antimicrobial Susceptibility Testing; Twenty-second Informational Supplement. Clinical Laboratory Standard Institute. Wayne, Pennsylvania, USA 2012; 32: 70–71.
12. Magiorakos AP, Srinivasan A, Carey RB, Carmeli Y and Falagas ME. Multidrug resistance, extensively drug resistance and pandrug-resistance bacteria: An international expert proposal for interim standard definitions for acquired resistance. Clinical Microbiology Infection, 2012; 18: 268-281.
13. Sham DF, Thomsberry C, Mayfield DC, Jones ME and Karlowsky JA. Multidrug resistant urinary tract isolates of *Escherichia coli*: Prevalence and patient demographics in United States in 2000. Antimicrobial Agents Chemotherapy, 2001; 45(5): 1402-1406.
14. Clinical Laboratory Standard Institute (CLSI) Performance Standards for Antimicrobial Susceptibility Testing; 20th informational Supplementd. Clinical and Laboratory Standard Institute. Wayne, Pennsylvania, USA; 2010, M100-S20.
15. Magiorakos AP, Srinivasan A, Carey RB, Carmeli Y, Falagas ME, Giske CG, Harbarth JF, Kahlmeter G, Olsson-Liljequist B, Paterson DL, Rice LB, Struelens MJ, Vatopoulos A, Weber JT and Monnet DL. Multidrug-resistant, extensively drug-resistant and pandrugresistant bacteria: an international expert proposal for interim standard definitions for acquired resistance. Clinical Microbiology and Infection, 2012; 18(3): 268-281.

© 2016 Global Journals Inc. (US)

16. Burke JP; Interscience Conference on Antimicrobial Agents and Chemotherapy Abstr Intersci Conf Antimicrob Agents Chemother Intersci Conf Antimicrob Agents Chemother. 1999 Sep 26-29; 39: 755 (abstract no. 623). bsiteat: http://gat.eway.nlm.nih.gov/MeetingAbstracts/ma? f= 1022459 61.html [accessed on 25 January 2009).
17. Larry GR, Michael LW and Melvin PW. Update on Detection of Bacteremia and Fungemia. Clinical Microbiology Reviews, 1997; 10(3): 444–465.
18. Subedi S, Maharjan J and Shrestha B. Antibiotic susceptibility test of *Klebsiella pneumoniae* and *K. oxytoca* isolated from different clinical samples and perform random amplified polymorphic DNA among *K. pneumoniae*. British Microbiology Research Journal, Science Domain international 2016; 12(6): 1-11.
19. Karki S, Rai GK and Manandhar R. Bacteriological analysis and antibiotic sensitivity pattern of blood cultures isolated in Kanti Children hospital. Journal of Nepal Paediatric Society, 2010; 30(2): 94-97.
20. Bhandari P, Manandhar S, Shrestha B and Dulal N. Etiology of bloodstream infection and antibiotic susceptibility pattern of the isolates. Asian journal of medical sciences 2016; 7(2): 71-75.
21. Kante M, Uma P, John MS and Naidu MP. Bacterial profile of blood stream infections and antibiotic susceptibility pattern of isolates. International Journal of Current Microbiology and Applied Sciences, 2014; 3 (12): 222-233.
22. Magiorakos AP, Srinivasan A, Carey RB, Carmeli Y, Falagas ME, et al. Multidrug resistance, extensively drug resistance and pandrug-resistance bacteria: An international expert proposal for interim standard definations for acquired resistance. Clinical Microbiology and Infection, 2012; 18: 268-281.
23. Labi AK, Obeng-Nkrumah N, Addison NO and Donkor ES. *Salmonella* blood stream infections in a tertiary care setting in Ghana. BioMed Central infectious disease, 2014; 14: 3857.
24. Sharma N, Koju R, Karmacharya B, Tamang MD, Makaju R, Nepali N, et al. Typhoid fever in Dhulikhel hospital, Nepal. Kathmandu University Medical Journal (KUMJ), 2004; 2: 188–192.
25. Bhatta D, Gaur A and Supram HS. Bacteriological profile of Blood Stream Infection among febrile patients attending a tertiary care centre of Western Nepal. Asian Journal of Medical Sciences, 2013; 4(3): 92–98.

© 2016 Global Journals Inc. (US)

This page is intentionally left blank

© 2016 Global Journals Inc. (US)

Global Journal of Medical Research: C
Microbiology and Pathology
Volume 16 Issue 1 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

A Preliminary Survey of Norovirus and Astrovirus Antigen in Diarrheic Stools of Children in Borno State, Nigeria

By Oyinloye, S.O., Jatau, E.D., Aminu, M. & Ella, E.E.

University of Maiduguri, Nigeria

Abstract- Background: Noroviruses and astroviruses are important agents of acute gastroenteritis among children. Acute gastroenteritis (AGE) is a major cause of morbidity and mortality in pediatric populations world-wide. Globally, an estimated 800 000 infants and young children die from diarrhea every year.

Aims: This was a cross-sectional study that included acute diarrheic children presenting in Specialist Hospital, Nursing Home Health Centre and Ngamdu pediatric clinic in Borno state with the aim of detecting norovirus and astrovirus antigen. Two hundred children whose parent/ guardian consented were enrolled in the study.

Methodology: Two hundred acute diarrheic and forty one nondiarrheic fecal samples were collected from children aged 5 or less between June 2013 – May 2014. Samples were screened for norovirus and astrovirus antigen using 3rd generation RIDASCREEN ELISA test kit. Demographic data of the children were obtained.

Results: All non-diarrheic stools were negative for both antigens whileof the two hundred diarrheic stools screened, a prevalence of 8% and 5% were obtained for norovirus and astrovirus respectively. The proportion of males (6/130) positive for norovirus antigen relative to female (10/70) was found to be significant ($p < 0.016199$). No significant difference was observed between male and female positive for Astrovirus ($p = 0.307574$). A significant prevalence of norovirus ($p = 0.00001$) and astrovirus ($p = 0.013321$) based on age group were obtained. Clinical sign and symptoms of infection with norovirus and Astrovirus between male and female showed no significant difference ($p=0.42018$).

Keywords: norovirus, astrovirus, diarrhea, borno state, nigeria.

GJMR-C Classification : NLMC Code: QW 51

APRELIMINARYSURVEYOFNOROVIRUSANDASTROVIRUSANTIGENINDIARRHEICSTOOLSOFCHILDRENINBORNOSTATENIGERIA

Strictly as per the compliance and regulations of:

© 2016. Oyinloye, S.O., Jatau, E.D., Aminu, M. & Ella, E.E. This is a research/review paper, distributed under the terms of the Creative Commons Attribution-Noncommercial 3.0 Unported License http://creativecommons.org/ licenses/by-nc/3.0/), permitting all non-commercial use, distribution, and reproduction in any medium, provided the original work is properly cited.

A Preliminary Survey of Norovirus and Astrovirus Antigen in Diarrheic Stools of Children in Borno State, Nigeria

Oyinloye, S.O. [α], Jatau, E.D. [σ], Aminu, M. [ρ] & Ella, E.E. [ω]

Abstract- Background: Noroviruses and astroviruses are important agents of acute gastroenteritis among children. Acute gastroenteritis (AGE) is a major cause of morbidity and mortality in pediatric populations world-wide. Globally, an estimated 800 000 infants and young children die from diarrhea every year.

Aims: This was a cross-sectional study that included acute diarrheic children presenting in Specialist Hospital, Nursing Home Health Centre and Ngamdu pediatric clinic in Borno state with the aim of detecting norovirus and astrovirus antigen. Two hundred children whose parent/guardian consented were enrolled in the study.

Methodology: Two hundred acute diarrheic and forty one non-diarrheic fecal samples were collected from children aged 5 or less between June 2013 – May 2014. Samples were screened for norovirus and astrovirus antigen using 3rd generation RIDASCREEN ELISA test kit. Demographic data of the children were obtained.

Results: All non-diarrheic stools were negative for both antigens whileof the two hundred diarrheic stools screened, a prevalence of 8% and 5% were obtained for norovirus and astrovirus respectively. The proportion of males (6/130) positive for norovirus antigen relative to female (10/70) was found to be significant ($p < 0.016199$). No significant difference was observed between male and female positive for Astrovirus ($p = 0.307574$). A significant prevalence of norovirus ($p = 0.00001$) and astrovirus ($p = 0.013321$) based on age group were obtained. Clinical sign and symptoms of infection with norovirus and Astrovirus between male and female showed no significant difference ($p=0.42018$).

Conclusion: Norovirus and Astrovirus are a significant aetiology of diarrhea in the study area. Measures to mitigate their sequelae are required to circumvent potential public health crises in future.

Keywords: norovirus, astrovirus, diarrhea, borno state, nigeria.

I. Introduction

Viral intestinal infections are the most common cause of acute infectious diarrhea in the pediatric group and accounted for approximately 70% of episodes of acute infectious diarrhea in children (1). Rotavirus, norovirus, adenovirus, and astrovirus are the recognized viral causes of pediatric gastroenteritis (2) and the World Health Organization (WHO) data showed that each child practically has viral diarrhea irrespective of race and socioeconomic status within the first 5 years of life and this has great economic burden for the system of public health services and all society (3)

Epidemiological studies on norovirus gastroenteritis have been conducted in countries such as the United States of America (4, 5), Finland (6), Australia (7), Italy (8), some developing countries such as Brasil (9), Iraq (10). In sub-Saharan Africa, molecular epidemiology studies of norovirus have also been performed in countries such as Malawi (41% prevalence), Ghana (16.4% prevalence), South Africa, Botswana, Cameroon and Burkina Faso (12% prevalence) (11-16). In a study in Lagos Nigeria, norovirus prevalence of 37.3% was obtained among children with acute gastroenteritis (17) while in Ife, Nigeria, norovirus single infections were found in 64.3% (9/14) of the norovirus positive diarrhoea samples (18). Also in Owo, Ondo state Nigeria, norovirus was found in 4/50 (8%) of the diarrheic children examined (19)

The first astrovirus infecting humans was described in 1975 (20). Since then, a total of 8 serotypes closely related to this original astrovirus ("classic human astroviruses" (HAstVs)) have been identified, all of which are believed to cause diarrhea. The prevalence of HAstV infection has been reported to be 2%–16% among children hospitalized with diarrhea and 5%–17% in community studies that used either Enzyme Immunosorbent Assay (EIA) or Reverse Transcriptase Polymerase Chain Reaction (RT-PCR) analysis (21-23). Seroprevalence studies indicate that most children are infected during the first 2 years of life (21, 24, 25). Previous studies in Nigeria show different prevalence of astrovirus. In a study in northwest Nigeria, 5% astrovirus positivity was reported (26) while others (17) and (27) reported 16% prevalence in Lagos and Nasarawa states respectively.

Diarrhea of viral aetiology is under reported in north east region of Nigeria. The aim of this study was to conduct a preliminary survey of norovirus and astrovirus antigen in acute diarrheic stool due to increasing epidemiological significance of viral gastroenteritis in north eastern region of Nigeria and dearth of published data on the existence or otherwise of diarrhea of norovirus and Astrovirus aetiology. It is hoped that the

Author α: Department of Microbiology, Faculty of Science, University of Maiduguri, Borno State, Nigeria. e-mail: faisam26@gmail.com
Author σ ρ ω: Department of Microbiology, Faculty of Science, Ahmadu Bello University, Zaria, Nigeria.

© 2016 Global Journals Inc. (US)

information generated in this study will serve as baseline data to inform the relevant authority in Nigeria of the health burden posed by these viruses.

II. Materials and Methods

a) Study Population

Samples were collected at random from children aged below 5years presenting with acute diarrhea at the In and Out Patient Departments and the Pediatric Wards of Specialist Hospital, Nursing Home Health Centre and Ngamdu pediatric clinic in Borno State, Nigeria.

b) Exclusion Criteria

Children above age of 5 years and those below 5 years, whose parents/guardians declined consent, were excluded from the study.

c) Inclusion Criteria

Diarrheic children aged below 5 years whose parents/guardians consented to participate in the research were included in the study. Diarrhea was defined as passage of three or more watery stool within the last 24-hour period.

d) Study Design

In this research, a cross sectional design was employed in order to allow for stool sample collection from every other child presenting at any of the selected hospital in the study area.

e) Ethical Approval

Ethical approval was sought and obtained from the Ethical Committees of the respective hospitals involved in the study.

f) Analyses of stool sample

Stool samples collected were assayed to detect norovirus, and astrovirus antigen using RIDASCREEN® ELISA Test Kit.

g) Detection of Norovirus, and Astrovirus by Enzyme Linked Immunosorbent Assay (ELISA)

i. *Sample preparation*

Each stool sample was prepared for analysis according to manufacturer's instruction:

One milliliter (1ml) RIDASCREEN® sample was placed in dilution buffer in a labelled test tube. Liquid stool was sucked up into a disposable pipette until it rose to just above the second mark (approx. 100 µl) and was suspended in the buffer which was placed in the tube beforehand. The stool suspension was homogenised either by suction and ejection from a disposable pipette or, alternatively, by mixing in a vortex mixer. The specimen was centrifuged at 5000 rpm (approx. 2300 – 2500 G) for 5 minutes and the resulting supernatant of the stool suspension was used.

ii. *ELISA Procedure*

One hundred microliter (100µl) of positive control, the negative control (specimen-dilution buffer diluent) and the stool supernatant were dispensed in the wells. One hundred microliter (100µl) of the biotin-conjugated antibody was added to the wells and incubated at room temperature (20 – 25 °C) for 60 minutes after mixing thoroughly (by lightly tapping on the edge of the plate), after this, the plates were washed 5 times using 300µl wash buffer each time using an automated machine. (The wells were emptied completely by knocking them out after each wash on a part of the absorbent paper which is dry and unused). One hundred microliter (100µl) of the streptavidin-peroxidase conjugate was added to the wells and incubated at room temperature (20 – 25 °C) for 30 minutes and washed as described above. One hundred microliter (100µl) of substrate was added to each well. Then the plate was incubated at room temperature (20°C - 25°C) for 15 minutes in the dark. The reaction was stopped by adding 50µl of stop reagent to each well. After mixing carefully (by lightly tapping the side of the plate) the extinction was measured at 450nm using a reference wavelength ≥ 600 nm (optional).

h) Evaluation and interpretation

Calculating the cut-off

In order to establish the cut-off, 0.15 extinction units are added to the measured extinction for the negative control.

Cut-off = Extinction for the negative control + 0.15

Test result

Samples are considered **positive** if their extinction is more than 10 % above the calculated cut-off.

Samples are considered **equivocal** and must be repeated if their extinction is within ± 10 % of the cut-off. If repeating the test with a fresh stool sample again yields a value in the grey range, the sample must be considered negative.

Samples with extinctions more than 10 % below the calculated cut-off must be considered **negative**.

III. Results

Of the two hundred diarrheic stool screened, a prevalence of 8% (16/200) and 5% (10/200) were obtained for norovirus and astrovirus respectively (Table 1). The proportion of males (6/130; 4.62%) positive for norovirus antigen relative to female (10/70; 14.29%) was found to be significant ($P < 0.016199$; Table 1) but not so for Astrovirus ($p = 0.307574$; Table 1). A significant prevalence of norovirus ($P = 0.00001$) and astrovirus ($P = 0.013321$) based on age group were obtained in this study (Table 1). Clinical sign and symptoms of infection with norovirus and Astrovirus between male and female showed no significant difference ($P = 0.42018$; Table 2).

© 2016 Global Journals Inc. (US)

Table 1 : Norovirus and Astrovirus distribution among children in Borno State according to Age and Sex

Variables	No. of Sample	Number Positive		p-value	
Age group (month)		Norovirus (%)	Astrovirus (%)	Norovirus	Astrovirus
1-6	13	0(0)	1(7.69)		
7-12	44	3(6.80)	1(2.27)		
13-24	49	6(12.20)	5(10.20)	0.00001	0.013321
25-36	35	4(11.40)	2(5.71)		
37-48	36	2(5.60)	1(2.78)		
49-60	23	1(4.30)	0(0.00)		
Total	**200**	**16(8.0)**	**10(5.0)**		
Sex					
Male	130	6(4.62)	5(3.85)	0.016199	0.307574
Female	70	10(14.29)	5(7.14)		
Total	**200**	**16(8.0)**	**10(5.0)**		

Table 2 : Norovirus and Astrovirus clinical sign/symptom in relation to sex

Sign and Symptom	No. of Sample	Norovirus positive		Astrovirus positive		p-value of M / F
		Male (M)	Female (F)	Male	Female	
Fever (F) only	67	1	2	1	1	0.42018
Vomiting (V) only	23	2	2	1	0	
Fever and Vomiting	39	1	4	3	3	
Abdominal Cramp	31	2	2	0	1	
Mucoid/bloody stool	40	0	0	0	0	

IV. Discussion

The results from the present study suggest that norovirus and astrovirus contribute significantly to the disease burden of childhood diarrhea in Borno state, Nigeria.

The norovirus prevalence of 8% (Table 1) is similar to 8% obtained in Owo, Nigeria (19) but lower than the prevalence (21%) found for children in the United States of America (28), and that reported in a pooled analysis of studies conducted in seven developing countries (12.1%), spanning from Malawi to Thailand to Peru (29). Also, the figure in the present study was lower than 37.5% found for children in Nigeria (17). The prevalence of astrovirus in Borno state of Nigeria found in this study was 5% (Table 1). It is within the prevalence range of 2%–16% of human astrovirus (HAstV) infection reported among children hospitalized with diarrhea and 5%–17% in community studies that used either EIA or RT-PCR analysis (21-23). The prevalence of astrovirus in this study was observed to be similar to 5% prevalence in northwest Nigeria (26); and 4.9% prevalence reported in Mexico, it was lower than 10.8% reported in the United States, and 16% prevalence in Nasarawa Nigeria (27).

These disparities in norovirus and astrovirus prevalence across different studies may have been caused by different reasons. One reason may be due to the period samples were collected relative to the duration of diarrhea. Norovirus and astrovirus shedding generally peak within the first week of illness but can last for nearly two months (30). This reflects how the duration of illness can affect the outcome of each study because samples collected after the peak period of viral shedding will, as expected, present a possible outright negative or false negative result thereby impacting on the prevalence to be reported. In this study, the duration of illness of patients was not recorded implying that some samples might have been collected perhaps after peak period of infection. This limitation is similar to a report in 2006 which neglected to list limits in the duration of illness (31). Another factor contributing to the disparities among results was the variance in age-based inclusion criteria among the various study populations. In the present study, children had to be less than five years of age, which is the age range most affected by diarrhea.

Sex stratification of the children sampled revealed that the proportion of males (6/130) positive for norovirus antigen relative to female (10/70) was found to be significant ($P < 0.016199$; Table 1). However, the prevalence of male (6/130: 4.62%) positive for norovirus antigen was found to be lower than that of female (10/70: 14.29%). This is contrary to previous study which had reported a greater male susceptibility rate. The greater susceptibility of male to norovirus infection had been attributed to genetic and immunological factors (32). Susceptibility to infection with human Astrovirus showed no association to gender ($P = 0.307574$; Table 1). Overall, the occurrences of infection between the sexes indicate that either male or female could be

© 2016 Global Journals Inc. (US)

infected. This information, if and when corroborated by other studies, can serve to guide possible vaccination policy in future to target children not more 5 years old.

In the present study, the age-based prevalence of norovirus in Borno state was found to be significant (P=0.0001; Table 1) affecting a greater proportion (9/16) of children less than or equal to age of 2 years. This finding is similar to that of other studies (28,33). Interestingly, 3/9 of these children were of age 7-12 month (Table 1). Possible reason for this observation is behavioural. Since the virus transmission is through fecal-oral route, fecal-contaminated items picked from the ground into the mouth by crawling children could serve as potential mechanical vector. For astrovirus, age-based prevalence was also significant (P=0. 0.013321; Table 1). We also observed in this study that the number of children less than or equal to age of 2 years positive for Astrovirus antigen were more (7/10) than children older than age 2 (3/10) (Table 1). Above reasons are applicable. However, from Table 1, for both norovirus and Astrovirus, the number of infected individual declined from age above 2 years. Boosted immunity and reduced tendency to consume soiled edibles might be attributable. This assertion has yet to be proven scientifically, though.

The most recurring clinical symptom observed among both sexes for norovirus and astrovirus (Table 2) respectively was fever with vomiting (5/39 and 6/39 respectively) though it was not significant between male and female (P=0.42018). Since vomiting (with or without fever) featured prominently as a clinical symptom of infection with both viruses, it implies that the sequelae will be dehydration. Although this study did not determine the association of norovirus and astrovirus diarrhea with dehydration, a previous study in Zaria, Nigeria, reported a rotavirus prevalence of 21% among those not dehydrated and 78.4% among those that were dehydrated (34). Norovirus and astrovirus are also implicated in diarrhea cases; therefore one could infer that the sequelae of infection among the children in this study would also be dehydration arising from vomiting. Hence, the ensuing dehydration warrants public enlightenment/education on the use of oral rehydration solution in order to reduce number of deaths due to possible severe dehydration.

V. Conclusion

This study has established the presence of norovirus and astrovirus in diarrheic stools of children in the Borno state. The prevalences obtained in this study reveal that, though it is under-reported, it could be a debilitating childhood disease due to dehydration. And since definitive diagnosis/examination is hardly done to ascertain causes of death in this part of the developing world, deaths which have been reported to be due to diarrhea related causes in neonates and children might have been caused by either or both of these enteric pathogens. Due to limited funds, we could not undertake immediate molecular characterization of the positive samples.

References Références Referencias

1. Imade PE, and Eghafona NO. Viral Agents of Diarrhea in Young Children in Two Primary Health Centers in Edo State, Nigeria. International Journal of Microbiology. 2015; 2015: 1-5.
2. Hart CA, Cunliffe NA, and Nakagomi OD. Diarrhea caused by viruses, in Manson's Tropical Diseases, G. C. Cook and A. L. Zumla, Eds., pp. 815–824, Saunders Elservier, Philadelphia, Pa, USA, 22nd edition, 2009.
3. Bulanova I, Feklisova K, and Titova I. Results of application of lacto-containing probiotics in viral diarrhea in young children. Childhood Infection. 2009; 2:58–60.
4. Vega E, and Vinjé J.Novel GII.12 Norovirus Strain, United States, 2009–2010. *Emerging Infectious Diseases*. 2011; 17: 1516-1518.
5. Blanton LH, Adams SM, Beard RS, Wei G, Bulens SN, and Widdowson MA. Molecular and epidemiologic trends of caliciviruses associated with outbreaks of acute gastroenteritis in the United States 2000–2004. *Journal of Infectious Diseases*. 2006; 193:413–421.
6. Pang XL, and Vesikari T. Human astrovirus-associated gastroenteritis in children under 2 years of age followed prospectively during a rotavirus vaccine trial. *Acta Paediatr* 1999; 88:532-536.
7. Kirkwood CD, and Bishop RF. Molecular detection of human calicivirus in young children hospitalized with acute gastroenteritis in Melbourne, Australia, during 1999. *Journal of Clinical Microbiology* 2001; 39:2722-2724
8. Colomba C, Saporito L, Giammanco GM, De Grazia S, Ramirez S, Arista S, Titone L. Norovirus and gastroenteritis in hospitalized children, Italy. Emerg. Infect. Dis. 2007; 9: 1389-1391.
9. Ribeiro LR, De Oliveira RS, Barreira DMPG, Saick KW, Leite JPG, Miagostovich MP, and Spano LC. Hospitalization due to norovirus and genotypes of rotavirus in pediatric patients, *stata of Espiritosanitomem. Institute Oswald Cruz*, Rio de Janeiro, 2008; 103: 201-206.
10. Al Mashhadani MN, Nakagomi O, Dove W, Ahmed, H, Nakagomi, T, Hart CA. and Cunliffe NA. Norovirus gastroenteritis among children in Iraqi Kurdistan. *Journal of Medical Virology*. 2008;80:506-509.
11. Dove W, Cunliffe NA, Gondwe JS, Broadhead RL, Molyneux ME. Detection and characterization of human caliciviruses in hospitalized children with acute gastroenteritis in Blantyre, Malawi. *Journal of Medical Virology* 2005; 77: 522–527.

© 2016 Global Journals Inc. (US)

12. Armah GE, Gallimore CI, Binka FN, Asmah RH, Green J, Ugoji U, Anto F, Brown DWG. and Gray JJ. Characterization of norovirus strains in rural Ghanian children with acute diarrhea. *Journal of Medical Virology*. 2006; 78: 1480-1485.
13. Mans J, de Villiers JC, du Plessis NM, Avenant T, Taylor MB. Emerging norovirus GII.4 2008 variant detected in hospitalised paediatric patients in South Africa. *Journal of Clinical Virology* 2010;49: 258–264.
14. Mattison K, Sebunya TK, Shukla A, Noliwe LN, Bidawid S. Molecular detection and characterization of noroviruses from children in Botswana. *Journal Medical Virology* 2010; 82: 321–324.
15. Ayukekbong J, Lindh M, Nenonen N, Tah F, Nkuo-Akenji T. Enteric viruses in healthy children in Cameroon: viral load and genotyping of norovirus strains. *Journal Medical Virology* 2011; 83: 2135–2142.
16. Nordgren J, Nitiema LW, Ouermi D, Simpore J, Svensson L. Host genetic factors affect susceptibility to norovirus infections in Burkina Faso. PLOS ONE. 2013; 8(7): 1-10
17. Ayolabi CI, Ojo DA, Armah GE, Akpan I and Mafiana CF. Detection and partial characterization of norovirus among children with acute gastroenteritis in Lagos, Nigeria. *International Journal of Medicine and Medical Sciences*. 2010; 2: 216-221.
18. Japhet MO, Adesina OA, Famurewa O, Svensson L, and Nordgren J. Molecular epidemiology of rotavirus and norovirus in Ile-Ife, Nigeria: high prevalence of G12P[8] rotavirus strains and detection of a rare norovirus genotype," Journal of Medical Virology. 2012;84:1489–1496
19. Babalola MO, Odaibo GN, Olaleye DO, and Alonge AO. Enteric Adenovirus and Norovirus Gastroenteritis among Under-5 years Children in Owo, Ondo State, Nigeria. British Journal of Medicine & Medical Research. 2015; 9: 1-9
20. Madeley CR, Cosgrove BP. Letter: 28 nm particles in faeces in infantile gastroenteritis. Lancet 1975; 19752: 451–452.
21. Maldonado Y, Cantwell M, Old M. Population-based prevalence of symptomatic and asymptomatic astrovirus infection in rural Mayan infants. *Journal of Infectious Disease 1998; 178: 334-9.*
22. Unicomb LE, Banu NN, Azim T, Astrovirus infection in association with acute, persistent and nosocomial diarrhea in Bangladesh. *Pediatrics Infectious Disease Journal* 1998; *17: 611-4.*
23. Pang XL, and Vesikari T. Human astrovirus-associated gastroenteritis in children under 2 years of age followed prospectively during a rotavirus vaccine trial.*Acta Paediatr* 1999; 88: 532-6.
24. Mitchell DK, Matson DO, Cubitt WD. Prevalence of antibodies to astrovirus types 1 and 3 in children and adolescents in Norfolk, Virginia. *Pediatrics Infectious Disease Journal. 1999*; 18: 249-54.
25. Kobayashi S, Kobayashi M, Araki K, Shinozaki T, Yanagawa Y. Antibody prevalence against astrovirus according to age groups. *Kansenshogaku Zasshi* 1999; 73: 578-83.
26. Aminu M, Esona MD, Geyer A. and Steele AD. Epidemiology of rotavirus and Astrovirus Infections in children in northwestern Nigeria. *Annals of African Medicine* 2008; 7: 168 – 174.
27. Kuta FA, Damisa D, Adabara NU, and Abdulsalam R. Prevalence of astrovirus infection in children in Nasarawa state, Nigeria. *Global Advanced Research Journal of Microbiology* 2014; 3: 102-105.
28. Payne DC, Vinjé J, Szilagyi PG. Norovirus and Medically Attended Gastroenteritis in U.S. Children. N. Engl. J. Med. 2013; 368: 1121–1130.
29. Manish M, and Patel MW. Systematic Literature Review of Role of Noroviruses in Sporadic Gastroenteritis. *Emerging Infectious Disease*. 2008; 14: 1224–1231.
30. Aoki Y, Suto A, Mizuta K, Ahiko T, Osaka K, Matsuzaki Y. Duration of norovirus excretion and the longitudinal course of viral load in norovirus-infected elderly patients. Journal Hospital Infection 2010; 75: 42–6.
31. Enweronu-Laryea CC, Sagoe KWC, Glover-Addy H., Asmah RH, Mingle JA, Armah GE. Prevalence of severe acute rotavirus gastroenteritis and intussusceptions in Ghanaian children under 5 years of age. *Journal of Infection in Developing Countries* 2012; 6:148–55.
32. Fischer TK, Viboud C, Parashar U. Hospitalizations and death from diarrhea and rotavirus among children < 5years of age in the United States, 1993-2003. J Infect Dis 2007; 195: 1117-1125.
33. Bucardo F, Nordgren J, Carlsson B, Pediatric norovirus diarrhea in Nicaragua. *Journal of Clinical Microbiology* 2008; 46: 2573–80.
34. Pennap G, Umoh J. The prevalence of group A rotavirus infection and some risk factors in pediatric diarrhea in Zaria, North central Nigeria. *African Journal of Microbiology Research*. 2010; 4: 1532-1536.

© 2016 Global Journals Inc. (US)

This page is intentionally left blank

© 2016 Global Journals Inc. (US)

Global Journal of Medical Research: C
Microbiology and Pathology
Volume 16 Issue 1 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

Prevalence, Isolation of Bacteria and Risk Factors of Mastitis of Dairy Cattle in Selected Zones of Oromia Regional States, Ethiopia

By Dinaol Belina, Yimer Muktar, Adem Hiko, Nateneal Tamerat, Tadesse Kebede, Tarekegn Wondimu & Jelalu Kemal

Haramaya University, Ethiopia

Abstract- A cross sectional study was conducted on a total of 471 cross and pure borana breed dairy cattle to determine prevalence of clinical and subclinical mastitis using CMT in selected districts of North Showa and Borana zones of pastoral area from April 2012 to February 2014. The overall mastitis prevalence was 237(50.3%). The cow level prevalence was 9.5% clinical and 40.7% were subclinical cases. Of 1884 quarters examined 10(0.5%) quarters were blind teats and quarters 550(50.2%) were showed mastitis. High score CMT positive milk sample were investigated using standard microbiological techniques. Identification of bacterial isolates revealed that 10 types of bacterial isolates were identified. The isolated bacteria were Staphylococcus aureus and CNS 20 (37.7%), *Diplococcus* spp 2 (3.8%), *Corynebacterium pseudotuberclosis* 3(5.8%), *Corynebacterium bovis* 1(1.7%), *Micrococcus* spp 1(1.9%), *Pseudomonas* spp 1(1.9%), *Bacillus* spp 1(1.9%), *E. coli* 3(5.7%) *Proteus* spp 1(1.9%). Different risk factors like parity number, farming hygiene, animal origin and husbandry type were considered. Hygienic conditions and husbandry type were the most important potential risk for mastitis.

Keywords: *CMT, bacterial culture, mastitis, prevalence, bovine, borana, north showa.*

GJMR-C Classification : *NLMC Code: QW 50*

PREVALENCEISOLATIONOFBACTERIAANDRISKFACTORSOFMASTITISOFDAIRYCATTLEINSELECTEDZONESOFOROMIAREGIONALSTATESETHIOPIA

Strictly as per the compliance and regulations of:

© 2016. Dinaol Belina, Yimer Muktar, Adem Hiko, Nateneal Tamerat, Tadesse Kebede, Tarekegn Wondimu & Jelalu Kemal. This is a research/review paper, distributed under the terms of the Creative Commons Attribution-Noncommercial 3.0 Unported License http://creativecommons.org/ licenses/by-nc/3.0/), permitting all non-commercial use, distribution, and reproduction in any medium, provided the original work is properly cited.

Prevalence, Isolation of Bacteria and Risk Factors of Mastitis of Dairy Cattle in Selected Zones of Oromia Regional States, Ethiopia

Dinaol Belina [α], Yimer Muktar [σ], Adem Hiko [ρ], Nateneal Tamerat [ω], Tadesse Kebede [¥], Tarekegn Wondimu [§] & Jelalu Kemal [χ]

Abstract- A cross sectional study was conducted on a total of 471 cross and pure borana breed dairy cattle to determine prevalence of clinical and subclinical mastitis using CMT in selected districts of North Showa and Borana zones of pastoral area from April 2012 to February 2014. The overall mastitis prevalence was 237(50.3%). The cow level prevalence was 9.5% clinical and 40.7% were subclinical cases. Of 1884 quarters examined 10(0.5%) quarters were blind teats and quarters 550(50.2%) were showed mastitis. High score CMT positive milk sample were investigated using standard microbiological techniques. Identification of bacterial isolates revealed that 10 types of bacterial isolates were identified. The isolated bacteria were *Staphylococcus aureus* and CNS 20 (37.7%), *Diplococcus* spp 2 (3.8%), *Corynebacterium pseudotuberclosis* 3(5.8%), *Corynebacterium bovis* 1(1.7%), *Micrococcus* spp 1(1.9%), *Pseudomonas* spp 1(1.9%), *Bacillus* spp 1(1.9%), *E. coli* 3(5.7%) *Proteus* spp 1(1.9%). Different risk factors like parity number, farming hygiene, animal origin and husbandry type were considered. Hygienic conditions and husbandry type were the most important potential risk for mastitis. Statistically, cattle from North Showa were highly infected than those from Borana zone ($p < 0.05$). Farmers and herd managers should give great attention for hygiene condition and husbandry type and further investigation should be conducted especially in the pastoral area.

Keywords: CMT, bacterial culture, mastitis, prevalence, bovine, borana, north showa.

I. Introduction

Ethiopia holds large potential for dairy development due to its large livestock population and the favorable climate for improved and high yielding breeds. Ethiopia has the largest livestock population among African countries (CSA, 2008).However, compared to other countries in Africa, Ethiopians consume less dairy products. Moreover, the quality and quantity of milk in the country deteriorates because of various causes. Given the considerable potential for generation of income and employment, the development of small holder dairy sector in Ethiopia, has a promising future and can contributes significantly to poverty alleviation improved nutrition in the country. According to Ahmed *et al.* (2007), milk production during the 1990s expanded at an annual rate of 3.0% compared to 1.63- 1.66% during the preceding three decades, with the expected growth in income, increased urbanization and improved policy environment (Kelay, 2002).The central highlands, mainly Selalle, are the major dairying areas, and as a result they are the main sources of milk for Addis Ababa, Ethiopia's capital and main urban population center, where 8% of its inhabitants live. Furthermore, farmers in Selalle are conscious of the milk market and produce milk for commercial sale, unlike the majority of Ethiopian farmers,who produce milk for home use. In Selalle farmers keep high-yield cross bred (zebu * Holstein) and Holstein dairy cattle mainly for milk production alongside native zebu breeds (Ameni *et al.,* 2007). Study by Kasim *et al.* (2012) indicated pure Borana breed are more productive than other local breeds in Ethiopia. However, milk production often does not satisfy the country's requirements due to a multitude of factors among them udder infection is the one (Erskine, 2001).

Mastitis is an inflammation of mammary gland, primary resulting from invasion of the mammary gland by pathogenic microorganisms through the teat canal resulting in physical, chemical, pathological and bacteriological changes in glandular tissues and milk. Many risk factors have been identified for clinical and subclinical mastitis in dairy animals such as breed, increased milk production, hygienic conditions, milking practices, age, parity, stage of lactation (Fox *et al*., 1995; Barnouin, and Chassagne, 2001).

The most common pathogen comprises contagious bacteria mainly *Staphylococcus aureus* and *Streptococcus agalactae* and environmental bacteria mainly coli forms and some species of streptococci that are commonly present in the environment (Radostitis *et al.,* 2007).Besides, mastitis may render milk unsuitable for human consumption or provide a mechanism for the spread of diseases like Tuberculosis, Streptococcal intoxication, Colibacillosis, streptococcal sore throat and Brucellosis to human

Bovine mastitis was reported as one of the most prevalent dairy health problems in Ethiopia including north Showa and Borana zones (Argaw and Tolosa,

Author α σ ρ ω ¥ § χ : College of Veterinary Medicine, Haramaya University, P.O. Box 138 Dire Dawa, Ethiopia.
e-mail: yimermktr21@gmail.com

© 2016 Global Journals Inc. (US)

2008). Yet, the information on the prevalence of sub clinical mastitis in the areas is lacking and what available is fragments of information from cases of clinical mastitis that has been presented to veterinary clinic for the treatment. Therefore, the objectives of this investigation were: to determine the prevalence and major risk factors associated with clinical and sub clinical mastitis at herd, cow and quarter level in small holder and pastoral area dairy cattle.

II. Materials and Methods

a) Study area

This study was carried out in North Shoa Zone of the Oromia Regional state in central Ethiopia and Borana pastoral and agro pastoral zone from April 2012 to February 2014. NorthShoa Zone is located in central Ethiopia 126 km north west of Addis Ababa in Oromia Regional state. It covers 1,174,500 hectare of land from which 40% is crop land, 25% is grazing land, 13% is forest and bush area, 7% is construction area and 15% is unproductive land. It's minimum and maximum temperatures vary from 11.5-29°C and 17.5-35°C, respectively. It gets bimodal rainfall that ranges from 651-1115mmi.e. from February-May (short rainy season) and from June- October (long rainy season) (North Shoa Agricultural Department, 2013).In particular Girarjarso, Debrelibanos and Wachale districts of North Showa were our study area.42% of the area is highland that is suitable for crop cultivation and livestock husbandry and the herd structure is characterized by a higher number of cows. Sample collection areas will be in 10 km radius of the milk collection units along the main road in both sides.

The study also conducted in Miyo District of Borana zone. The district is located at 717 Kms south of Addis Ababa. This district is characterized by pastoral and agro-pastoral production systems. The livestock species reared within the district are cattle (61,023), goat (72,224), sheep (14,567), camel (15,672), equines (12,613) and poultry (11,236). The rainfall pattern is bimodal in nature with average annual rainfall around 700mm. The average annual temperature is 22 °C. The altitude of the district ranges from 1300-1520 m.a.s.l (CSA, 2008).

b) Study Population

The study were conducted on a total of 144 heads of cross (Zebu and HF) breeds(of lactating cows kept under small holder dairy herds kept under extensive, semi- intensive and intensive husbandry practice in Salale of North Showa, and on327 pure Borana breed characterized by both meat and milk production merits.

c) Study design

A cross sectional study was conducted on small scale holder dairy farms in North Showa, and pastoral and agro pastoral area in Borana of Southern Ethiopia. Data on each cow was collected in a format designed for this purpose. The data sheet mainly focused to address associated risk factors with the occurrence of bovine mastitis. Risk factors considered were cow history, housing system, milking practice, hygiene, parity number, stage of lactation, husbandry type and other management practices in the study area.

d) Sample Size determination

The sample size was calculated according to the formula given by Thrusfield (2007). In Salale of North Showa it is calculated by taking (89.54%) prevalence from previous report by Argaw and Tolosa (2008).In Miyo of Borana considering the previous prevalence 59.3% in and around Yabello district (Kasim *et al.,* 2012), a total of 374 lactating dairy cows were proposed to be sampled but, attributable to the2010/11 drought shock of the zone only 327 heads of cows were included.

e) Sampling Procedure

To include cows from small scale holder dairy farms in selected districts of North Showarandom sampling method was employed to select the individual dairy cow. Selection was done by judgment mainly following accessibility to the main road in 10 km radius which is supplying their milk to eight primary farmer milk cooperatives in the three study districts. In Miyo of Borana purposive sampling was used to test lactating dairy cows available within the district due to the fact that the number of cows within the district are limited as a result 2010/11 drought shock in the zone.

f) Study Methodology

i. *Clinical Inspection of the Udder*

The clinical inspection of the udder was done in the following way. The udder was first examined visually and then by palpation to detect fibrosis, inflammatory swellings, visible injury, atrophy of the tissue, and swelling of supra mammary lymph nodes. The size and consistency of mammary quarters were inspected for the presence of any abnormalities, such as disproportional symmetry, swelling, firmness, and blindness. Information relating to the previous health history of the mammary quarters and causes of blindness was obtained from interviews with owners. Viscosity and appearance of milk secretion from each quarter were examined for the presence of clots, flakes, blood, and watery secretions (Quinn *et al.,* 2002).

g) Laboratory test

i. *California mastitis test (CMT)*

The California mastitis reagent was used to screen cows with subclinical mastitis when milk sample is collected according to the procedures of National Mastitis Council (NMC, 1999). Milk sampled according to National Mastitis Council, (NMC, 1999) was subjected to California mastitis reagent to screen subclinical

© 2016 Global Journals Inc. (US)

mastitis described by (Quinn *et al.,* 2002).This test is based on increased number of leucocytes and increased alkalinity in milk due to mastitis (as an indirect measurement of leucocytes). 0.5ml of milk from each quarter is taken in plastic peddle cups and added to equal quantity of CMT reagent solution and mix well by circular movement of peddles mixed on a horizontal plane. The result of the test indicated on the basis of gel formation .Depending on clinical inspection and CMT results, cases were categorized as either positive or negative. Positive cases were further categorized as clinical and sub-clinical mastitis. The interpretation (grades) of the CMT was evocated and the results was graded as 0 for special chemical concentrated commercially prepared negative and trace 1, 2 and 3, for positive (Quinn *et al.,* 2002).

h) Microbial investigation of mastitis

i. *Milk sample collection*

The milk sample was taken from cows not treated previously with either intra mammary or systematic antimicrobials agents. For good collection of sample the teat was wiped thoroughly with 70% ethyl alcohol. Procedures for collecting milk sample were according to (NMC, 1999; Quinn *et al*., 2002). Strict aseptic procedures were used when collecting milk samples in order to prevent contamination with the microorganisms present on the skin of cow's flanks, udder and teats, on the hands of the sampler, and in the barn environment. Teats towards sample collection were taken first and then the far ones. The first 3-4 streams of milk were discarded. The collecting vial was as near horizontal as possible and by turning the teat to a near horizontal position. The milk sample was held in an ice box and transported immediately to the laboratory for culturing.

i) Bacteriological isolation and biochemical characterization

Culturing of milk sample was performed according to microbiological producers of (Quinn *et al*., 2002 and Radostits *et al*., 2007) for the diagnosis of bovine mastitis. Briefly, a loopful of milk sample collected from each infected quarter was inoculated separately on to MacConkey agar and blood agar base enriched with 5% defibrinated sheep blood. The inoculated plates were then incubated aerobically at 37^0C for 24 to 48 hours. Identification of the bacteria on primary culture was made on the basis of colony morphology, hemolytic characteristics, Gram stain reaction including shape and arrangements of the bacteria, catalase and O-F tests. Staphylococci were identified based on Catalase test, growth characteristics on Mannitol salt agar and purple agar and tube coagulase test. Gram negative isolates grown on MacConkey agar were identified based on growth characteristics on MacConkey agar, Oxidase reaction, Catalase test, triple sugar iron (TSI) agar ,the "IMViC" (indole ,methyl red, Voges-Proskaur, and citrate) test (Quinn *et al.,* 2002).

j) Data Management

The collected data about history, clinical inspection, CMT, and result of bacterial culture were evaluated using SPSS soft- ware (SPSS 20.0 version) with Chi-square ($\chi 2$) and $p < 0.05$ was considered statistically significant.

III. Results

Of a total 471 examined lactating cows the overall prevalence of mastitis in the study areas was 237 (50.3%).The result showed that the prevalence of clinical and subclinical mastitis were 9.5 % and 40.7%, respectively. The result also indicated prevalence of bovine mastitis in north Showa zone is higher as compared to the Borana zone (Table.1).

Table 1 : Prevalence of Bovine Mastitis in Borana and North Showa zone

Variable	Levels	No. examined	Prevalence (%)			P value
			Clinical	Sub clinical	Subtotal	
Zone	Borana	327	40(12.2)	70(21.4)	110(33.6)	0.00
	North Showa	144	5(3.5)	122(84.7)	127(88.2)	
Districts/PA	Mio	68	10(14.7)	16(23.5)	26 (38.2)	0.00
	Baha	80	6(7.5)	20(25.0)	26(32.5)	
	Boku-luboma	83	12(14.4)	14(16.9)	26(31.3)	
	Hiddi	35	3(8.6)	5(14.3)	8(22.9)	
	Dhokisu	61	9(14.7)	15(24.6)	24(39.3)	
	G/ Jarso	54	2(3.7)	50(92.60	52(96.3)	
	D/Libanos	49	2(4.1)	45(91.8)	47(95.9)	
	Wuchale	41	1(2.4)	27(65.9)	28(68.3)	
	Total	471	45(9.5)	192(40.7)	237(50.3)	

© 2016 Global Journals Inc. (US)

Out of 1884 quarters examined 10(0.5%) quarters were blind, leaving 1884 functional quarters. The prevalence of mastitis on quarter bases was 550 (29.2%). The result showed that higher infection rate in hindquarters as compared to the front quarters (Table 2).

Table 2 : The Prevalence of Mastitis at quarter level

Quarter	No. examined	No. positive (%)			No. blind
		clinical	subclinical	Sub total	
Right front	471	24(17.8)	111(82.2)	135(28.9)	4(0.8)
		X^2=199.9,	P=0.00		
Right back	471	23(16.3)	118(83.7)	141(30.1)	2(0.4)
		X^2=202.0,	P=0.00		
Left front	471	23(17.2)	111(82.8)	134(28.6)	3(0.6)
		X^2=192.3,	P=0.00		
Left back	471	21(15)	119(85)	140(29.8)	1(0.2)
		X^2=203.3,	P=0.00		
Total	1884	91(16.5)	459(83.5)	550(29.2)	10(0.5)

The result showed that the effect of lactation stage and parity number were statistically insignificant (P>0.05) on the prevalence of bovine mastitis. However, husbandry type and hygienic condition were statistically have significant differences on the infection prevalence (P=0.00) (Table. 3).

Table 3 : prevalence of bovine mastitis based on lactation stage,husbandry practice, hygienic condition and parity number

Risk factors		Result				P value
		No. examined	No. positive (%)			
			clinical	subclinical	Sub total	
Lactation Stage	Early	141	17(21.5)	62(78.5)	79(56.0)	
	Mid	111	7(14.6)	41(85.4)	48(43.2)	0.13
	Late	219	21(19.1)	89(80.9)	110(50.2)	
Husbandry	Extensive	17	0(0)	13(100)	13(76.5)	
	Semi Intensive	84	3(3.9)	74(96.1)	77(91.6)	
	Intensive	43	2(5.4)	35(94.6)	37(86.0)	0.00
	Pastoral	232	28(35.4)	51(64.6)	79(34.0)	
	Agropastoral	95	12(38.7)	19(61.3)	31(32.6)	
Hygiene	Good	74	2(3)	66(97)	68(91.9)	
	Medium	52	1(3.2)	30(96.8)	31(59.6)	0.00
	Poor	345	42(30.4)	96(69.6)	138(40.0)	
Parity number	1-3	338	38(21.7)	137(78.3)	175(51.8)	
	4-6	109	5(10.40	43(89.6)	48(44.0)	0.26
	>6	24	2(14.3)	12(85.7)	14(58.3)	
	Total	471	45(19)	192(81.0)	237(50.3)	

From the CMT positive samples, 63 milk samples were cultured and 53 bacteria were cultured on blood, nutrient agar for gram positive bacteria and Macconkey agar for gram negative aerobically. *S. aureus* and CNS (coagulase negative staphylococci), were the most isolated followed by *C. pseudotuberclosis* and *E. coli* and the other such as *Diplodocus* Spp., *C. bovis, Micrococcus* Spp., *Bacillus* Spp., and *Pseudomonas* Spp. were rarely isolated as causative agents of mastitis. (Table 4).

Table 4 : Prevalence of isolated bacteria in the study areas in (2012-2014).

Bacteria	Frequency	Proportion%
S. aureus	20	37.7
CNS	20	37.7
Diplococcus species	2	3.8
C.pseudotuberclosis	3	5.7
C. bovis	1	1.9
Micrococcus species	1	1.9
Pseudomonas	1	1.9
Bacillus species	1	1.9
E .coli	3	5.7
Proteus species	1	1.9

© 2016 Global Journals Inc. (US)

IV. Discussion

Mastitis prevalence at cow level was found 50.3% which is highly disagreed with the report of Kifle and Tadele (2000) who reported 89.5% from North showa. This finding was also lower than the reports of Tariku *et al* (2011), Mekibib *et al* (2010) and Matios *et al* (2008) which were reported 75.2%, 71.3% and 65.5% respectively. However, it is slightly comparable with the finding of Birru (1989) who reported 43.5% from of Ethiopia. Mastitis is a multi –factorial disease and this difference may be due to herd size, agro- ecological and different managemental systems. In addition the present study shows the prevalence of mastitis is statistically higher ($p<0.05$) in north Showa than in Borana zone. As compared to the other districts/PAs of the study areaprevalenceof mastitis is highly important in w/jarso of the North showa and this may due to poor sanitation of the barn floor and use of organic bedding. Generally, the overall prevalence of mastitis reported from North Showa in the present study was higher than most of reports in the country and this could be due to major farmer depending on cross breed dairy cattle, absence of balanced diet feeding and lack of awareness about the disease.

From 1884 examined quarters, 550(50.2%) were CMT positive and 10(0.5%) quarters were blind. This finding is lower than that obtained by Kifle and Tadele (2000) and Birehanu (2008) as they reported 63.1% and 52.4% respectively at quarter level. Mekibib *et al.* (2010) reported that the prevalence of blind teat was 14% which is far higher than the present finding.

The prevalence of clinical mastitis at cow level was 9.5% and this is comparable with the reports of Molalegne *et al* (2010), Bedada and Hiko (2011) and Demelash *et al* (2005) who reported 10.3%, 10% and 11.9% respectively in different parts of Ethiopia. The current finding is little higher than the reports of Husien *et al* (1999) and Bishi (1998) who reported 5.7% and 5.3% respectively in different parts of the country. This may be due to concurrent disease involvement, interaction of several risk factors relating with animal and virulence of causative organism. In our study the rate of sub clinical mastitis is higher in pastoral production system than agro-pastoral (Table 2) attributable to higher number of animals in this system than agro-pastoral.

In this study, the prevalence of subclinical mastitis was 40.7% and this is lower than that reported by Argaw and Tolosa (2008) 89.5%. In addition, it is nearly similar to that founded by The finding is Bishi (1998) and Ahmed *et al*. (2007) However, our finding is higher than Mekibib *et al* (2010) who reported 25.2%. The high prevalence of subclinical mastitis may be due to improper milking hygiene, poor housing system, lack of post milking teat dipping.

Lactation stage showed statistically insignificant effect on the occurrence of mastitis $P>0.05$) which contradicts with the report of Birru (1989).Parity number has also no difference for occurrences of bovine mastitis.

In our study husbandry and hygiene showed statistically significant effect on the prevalence of mastitis ($p<0.05$). This could be due to different farming system, managemental practice and may be attributed to occurance of contagious mastitis and inability of control and physiology effect.

From the isolated bacteria the most dominant in the study area were CNS and *staphylococcus aureus* (37% for each) and the predominant causes of clinical and sub clinical mastitis in the area. This finding is little different from the finding of Molalegn *et al* (2010) who reported, (51.9%) of CNS. This study is compatible with the study of Mekibib *et al* (2010) and Abdella (1996) who reported (47.1%) and (31%) of *S. aures* respectively. In addition Tariku *et al.* (2011) reported 39.44% of *staphylococcus* species responsible for mastitis. However, the current finding is much lower than Workineh *et al.* (2002) who reported 70.5% of *Staphylococcus* species. The difference might be resulted from lack of effective udder washing and drying, inter cow hand washing and disaffection in the route of the area.

The other isolated bacteria were *E. coli* and *C. pseudotuberclosis (*5.56 % for each) and *Diplococcus* species 3.8%. This finding is lower than Hunder *et al.* (2005) who reported 14.2% of *C. Pseudotuberclosis* around Sebeta. Rarely isolated bacteria were *C. bovis, Baccilus spp, Micrococcus spp, Proteus spp* and *Pseudomonas spps* with equal ration of 1.9% for each. *B. cereus* and *B. subtilis* are saprophytic *Bacillus species* and the only mastitis causing pathogens (Radostits *et al.,* 2007).This result is in agreement with Tariku *et al* (2011) who reported (3.3%) *Micrococcus spp* and (2%) *C. bovis.*

In the current study the hind quarter have high mastitic prevalence and this may due to the hindquarter is more prone surface when the animal lay down touches hind leg in contact with contagious bacteria and to greater production capacity of hind quarter (Radostits*et al.,* 1994), likelihood of fecal accumulation, environmental contamination and difficulty of cleaning.

V. Conclusion and Recommendation

The overall prevalence of cattle mastitis is high as compared to most of previous reports in different parts of Ethiopia. Hygienic conditions and husbandry type were the most important potential risk for mastitis. The major isolated bacteria in this study were CNS and *S. aureus.* Farmers and herd manager are only concerned with clinical from of mastitis and often are unawareness of the status of subclinical infection in the

© 2016 Global Journals Inc. (US)

herd. The farmers have no enough understanding about effect of sanitation on the occurrence of the disease. Relaying on the above conclusion the following recommendation is for warded:

- Farmers and herd managers should give great attention for hygiene condition and husbandry type
- Periodic monitoring of infection status of the udder should be undertaken and positive animals should be treated using appropriate drug
- Careful milking practice and hygienic condition should be applied and the use of dry towel or sponge at least for each cow should be practiced
- Contaminated washing water and inappropriate bedding material that predispose to animals to mastitis should be avoided.
- Further investigation should be conducted on the area

Conflicts of interest

The authors have none to declare

Références

1. Abdella M (1996). Bacterial cause of bovine mastitis in Wondogenet, central Ethiopia. Vet.Med. Res. Hith .journal of veterinary medicine serious, 43(6) Pp 374-384
2. Ahmed A, Rady M, Mahammed S (2007). Epidemiological study on sub clinical mastitis in dairy cow in Assiut Governor at. *Adv. J. Vet. Med.* **2** (10): 373-380.
3. Ameni G, Aseffa A, Engers H, Young D, Gordon S, Hewinson G, Vorder Meier M (2007). High prevalence and increased severity of pathology of bovine tuberculosis in Holsteins compared to zebu breeds under field cattle husbandry in central Ethiopia. *Clinical and Vaccine Immunology* **14**: 1356-1361.
4. Argaw K, Tolosa T (2008). Prevalence of sub clinical mastitis in small holder dairy farms in Selale, North Shoa Zone, Central Ethiopia. *The Inter Journal of Veterinary Medicine*. **5**(1):74-93.
5. Barnouin J, Chassagne M (2001). Predictive variables for the occurrence of early clinical mastitis in primiparous Holstein cows under field conditions in France. *Can. Vet. J.***42**: 47-53.
6. Bedada BA, Hiko A (2011). Mastitis and antimicrobial susceptibility test at Asela, Oromia region, Ethiopia. Department of Veterinary Medicine, Epidemiology, Microbiology and Public Health, FVM, 10: 93.
7. Birehanu A (2008). Risk factor, isolation and identification of major bacteria and antimicrobial susptebility test in and around Asela. DVM thesis, Haramaya University, Ethiopia.
8. Birru G (1989). Major bacteria causing bovine mastitis and their sensitivity to common anti.
9. Bishi A (1998). Cross –sectional and longitudinal prospective study of bovine clinical and subclinical mastitis in per urban dairy production system in Addis Ababa Ethiopia MSc. thesis. Faculty of Vet Medicine, Addis Ababa University School of graduate study.
10. CSA (centeralstatististics authority) (2008).Federal Democratic Republic of Ethiopia: Agiricultural sample enumeration statistical report.
11. Demelash B, Etana D, Fekadu B (2005). Prevalence and risk factor of mastitis in lactating dairy cows in Southern Ethiopia Awassa College of agriculture, Debub University, Awassa, Ethiopia. *J. vet. med.* **3** (3): 35-39.
12. Erskine RJ (2001). Mastitis control in dairy farms. In: Radostits, O.M. (eds), herd health, food animal production medicine, 3rd ed. W.B. Saudres. Company, Philadelphia, Pennsylvania pp 397-432.
13. Fox LK, Chester ST, Hallberg JW, Nickerson SC, Pankey JW, Weaver LD (1995). Survey of intramammary infections in dairy heifers at breeding age and first parturition. *J. Dairy Sci.*, **78**: 1619-1628.
14. Hunder S, Adem Z, sitaayehu A (2005). Dairy cattle mastitis in and around sebeta, Ethiopia. *Res. vet. Med.* 3-4.
15. Husien N, Yehualashet T, Tilahun G (1999). Prevalence of mastitis in different local and exotic breed of milking cows. *Ethio. J. Agri. Sc.***16**: 53-60.
16. Kasim G, Bedane A, Yohannis T, Habtamu T, Asseged B, Demelash B (2012).Study on Prevalence and Risk Factors of Bovine Mastitis in Borana Pastoral and Agro-Pastoral Settings of Yabello District, Borana Zone, Southern Ethiopia
17. Kelay B (2002). Analysis of dairy cattle breeding practices in selected areas of Ethiopia. Zurwrlangung des akadamichey, Grades doctor rerumagrculturam/DR.reragr, cingerichtetader land-wirtschaft.
18. Kifle A, Tadele T (2000). Prevalence of subclinical mastitis in small holder dairy farms in selale, north Showa Zone, central Ethiopia. Ministry of agricultural and department of wild life conservation and College of Agriculture and Veterinary Medicine Jimma Universty; **5**(1): 34-41.
19. Matios L, Tadele T, Worku T (2008). Prevalence and major bacteria cause of bovine mastitis in Asella, South Eastern Ethiopia. *Tropl. Anim. health and prod*. **41**(7): 1525-1530.
20. Mekibib B, Furgasa H, Abunna F, Megersa B, Regassa A (2010). Bovine mastitis prevalence, risk factor and major pathogen in dairy farm of Holota town, Center of Ethiopia. **3**(9): 397-403.
21. Molalegn B, Arega T, Tafera N, Tadele T (2010). Study on bovine mastitis in dairy farm of Bahirdar. IDO: 919231; 2912-2917.

© 2016 Global Journals Inc. (US)

21. Molalegn B, Arega T, Tafera N, Tadele T (2010). Study on bovine mastitis in dairy farm of Bahirdar. IDO: 919231; 2912-2917.
22. NMC (1999). Current Concept in bovine mastitis National mastitis Council (NMC). 3rd .1840 Wilson-blud, Arlinton, VA 22201.
23. Quinn PC, Arter M, Markey B, carter G (2002). Clinical veterinary microbiology Harcaunt publisher Virginia Pp.457-460.
24. Radostits O, Blood D, Gay C (1994). Mastitis in Veterinary Text Took of the disease of cattle, sheep, goats and horse 8th ed. Baillir Tindal. Londo, Pp 563-614.
25. Radostits OM, Gay CC, Blood DC, Hinchlif KW (2007). Mastitis. In: Veterinary medicine 9thed, Harcourt Ltd, London, 174-758.
26. Smith M, Sherman D, (1994). Goat medicine 1st ed. Williams and Wilkins Awaverly Company, USA: PP 465-487.
27. Tariku S, Jamal H, Molalegne M (2011). Prevalence and susceptibility assay of Staphylococcus aureus isolation from mastitis in dairy farm of Jimma town, South West
28. Thrusfield M (2007). Veterinary Epidemiology, UK, Black well science, 3rd ed., 178-197.
29. Workineh S, Bayleyeng M, Mekonin H, Porgieter L (2002). Prevalence and etiology of mastitis in cow from two major Ethiopia dairy farms. *Trop. Animal. health and prod*. **34**: 19-25.

© 2016 Global Journals Inc. (US)

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.

© Copyright by Global Journals Inc.(US)| Guidelines Handbook

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 whilepublishing 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.

© Copyright by Global Journals Inc.(US) | Guidelines Handbook

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

A

Athrombogenic · 3, 6

B

Barnouin · 37, 42
Bullae · 1, 9

C

Chemotactic · 21
Colibacillosis · 37
Corynebacterium · 37

D

Diplococcus · 37, 40, 41
Disfibrinogenemiya · 1

E

Eosinophils · 16
Erysipelas · 1, 2, 11

F

Fisiologia · 12

G

Girarjarso · 38

H

Haptoglobin · 1, 3, 7

K

Krovoobraschenia · 12

L

Leukotrienes · 21
Lymphostasis · 3

N

Neutrophil · 1, 6, 9
Nosocomial · 25, 36

O

Onychomycosis · 2

P

Pentoxifylline · 9
Pneumoniae · 28, 29, 30
Pyogenes · 24, 26, 27, 28, 29

S

Septicaemia · 24
Sheremetiev · 3, 11

T

Thrombophlebitis · 1, 24
Tryptamine · 22

W

Wilcoxon · 2, 4

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

© Global Journals