Optimization of Citric Acid
Potent Phagocytic Microorganism

Highlights
Debre Markos Referral Hospital
Expression of Basal Cytokeratins

Discovering Thoughts, Inventing Future

VOLUME 17 ISSUE 2 VERSION 1.0
© 2001-2017 by Global Journal of Medical Research, USA
# Editorial Board

**Global Journal of Medical Research**

<table>
<thead>
<tr>
<th><strong>Dr. Apostolos Ch. Zarros</strong></th>
<th><strong>Dr. William Chi-shing Cho</strong></th>
</tr>
</thead>
<tbody>
<tr>
<td>DM, Degree (Psycho) holder in Medicine, National and Kapodistrian University of Athens</td>
<td>Ph.D., Department of Clinical Oncology, Queen Elizabeth Hospital, Hong Kong</td>
</tr>
<tr>
<td>MRes, Master of Research in Molecular Functions in Disease, University of Glasgow FRNS</td>
<td>Fellow, Royal Numismatic Society Member, European Society for Neurochemistry Member, Royal Institute of Philosophy Scotland, United Kingdom</td>
</tr>
<tr>
<td><strong>Dr. Alfio Ferlito</strong></td>
<td><strong>Dr. Michael Wink</strong></td>
</tr>
<tr>
<td>Professor Department of Surgical Sciences, University of Udine School of Medicine, Italy</td>
<td>Ph.D., Technical University Braunschweig, Germany</td>
</tr>
<tr>
<td><strong>Dr. Jixin Zhong</strong></td>
<td><strong>Head of Department Institute of Pharmacy and Molecular Biotechnology, Heidelberg University, Germany</strong></td>
</tr>
<tr>
<td>Department of Medicine, Affiliated Hospital of Guangdong Medical College, Zhanjiang, China, Davis Heart and Lung Research Institute, The Ohio State University, Columbus, OH 43210, US</td>
<td><strong>Dr. Pejcic Ana</strong></td>
</tr>
<tr>
<td><strong>Rama Rao Ganga</strong></td>
<td>Assistant Medical Faculty Department of Periodontology and Oral Medicine University of Nis, Serbia</td>
</tr>
<tr>
<td>MBBS</td>
<td><strong>Dr. Ivandro Soares Monteiro</strong></td>
</tr>
<tr>
<td>MS (University of Health Sciences, Vijayawada, India)</td>
<td>M.Sc., Ph.D. in Psychology Clinic, Professor University of Minho, Portugal</td>
</tr>
<tr>
<td>MRCS (Royal College of Surgeons of Edinburgh, UK) United States</td>
<td><strong>Dr. Sanjay Dixit, M.D.</strong></td>
</tr>
<tr>
<td><strong>Dr. Izzet Yavuz</strong></td>
<td>Director, EP Laboratories, Philadelphia VA Medical Center Cardiovascular Medicine - Cardiac Arrhythmia Univ of Penn School of Medicine Web: pennmedicine.org/wagform/MainPage.aspx?</td>
</tr>
<tr>
<td>MSc, Ph.D., D Ped Dent.</td>
<td><strong>Antonio Simone Laganà</strong></td>
</tr>
<tr>
<td>Associate Professor, Pediatric Dentistry Faculty of Dentistry, University of Dicle Diyarbakir, Turkey</td>
<td>M.D. Unit of Gynecology and Obstetrics Department of Human Pathology in Adulthood and Childhood “G. Barresi” University of Messina, Italy</td>
</tr>
<tr>
<td>Name</td>
<td>Title</td>
</tr>
<tr>
<td>-----------------</td>
<td>-------------------------------------------------------------------------------------------</td>
</tr>
</tbody>
</table>
| Dr. Han-Xiang Deng | MD, Ph.D  
Associate Professor and Research Department  
Division of Neuromuscular Medicine  
Davee Department of Neurology and Clinical Neurosciences  
Northwestern University Feinberg School of Medicine  
Web: neurology.northwestern.edu/faculty/deng.html |                                                                      |                                                                                           |
| Dr. Pina C. Sanelli | Associate Professor of Radiology  
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  
Web: weillcornell.org/pinasanelli/ |                                                                      |                                                                                           |
| Dr. Roberto Sanchez | Associate Professor  
Department of Structural and Chemical Biology  
Mount Sinai School of Medicine  
Ph.D., The Rockefeller University  
Web: mountsinai.org/ |                                                                      |                                                                                           |
| 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  
Web: uphs.upenn.edu/ |                                                                      |                                                                                           |
| Dr. Feng Feng | Boston University  
Microbiology  
72 East Concord Street R702  
Duke University  
United States of America |                                                                      |                                                                                           |
| Dr. Seung-Yup Ku | M.D., Ph.D., Seoul National University Medical College, Seoul, Korea Department of Obstetrics and Gynecology  
Seoul National University Hospital, Seoul, Korea |                                                                      |                                                                                           |
| Dr. Hrushikesh Aphale | MDS- Orthodontics and Dentofacial Orthopedics.  
Fellow- World Federation of Orthodontist, USA. |                                                                      |                                                                                           |
| Santhosh Kumar | Reader, Department of Periodontology,  
Manipal University, Manipal |                                                                      |                                                                                           |
<p>| Dr. Aarti Garg | Master of Tropical Veterinary Sciences, currently pursuing Ph.D in Medicine |                                                                      |                                                                                           |</p>
<table>
<thead>
<tr>
<th>Name</th>
<th>Qualifications</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sabreena Safuan</td>
<td>Ph.D (Pathology) MSc (Molecular Pathology and Toxicology) BSc (Biomedicine)</td>
</tr>
<tr>
<td>Arundhati Biswas</td>
<td>MBBS, MS (General Surgery), FCPS, MCh, DNB (Neurosurgery)</td>
</tr>
<tr>
<td>Getahun Asebe</td>
<td>Veterinary medicine, Infectious diseases, Veterinary Public health, Animal Science</td>
</tr>
<tr>
<td>Rui Pedro Pereira de Almeida</td>
<td>Ph.D Student in Health Sciences program, MSc in Quality Management in Healthcare Facilities</td>
</tr>
<tr>
<td>Dr. Suraj Agarwal</td>
<td>Bachelor of dental Surgery Master of dental Surgery in Oromaxillofacial Radiology. Diploma in Forensic Science &amp; Oodntology</td>
</tr>
<tr>
<td>Dr. Sunanda Sharma</td>
<td>B.V.Sc.&amp; AH, M.V.Sc (Animal Reproduction, Obstetrics &amp; gynaecology), Ph.D.(Animal Reproduction, Obstetrics &amp; gynaecology)</td>
</tr>
<tr>
<td>Osama Alali</td>
<td>PhD in Orthodontics, Department of Orthodontics, School of Dentistry, University of Damascus. Damascus, Syria. 2013 Masters Degree in Orthodontics.</td>
</tr>
<tr>
<td>Shahanawaz SD</td>
<td>Master of Physiotherapy in Neurology PhD- Pursuing in Neuro Physiotherapy Master of Physiotherapy in Hospital Management</td>
</tr>
<tr>
<td>Prabudh Goel</td>
<td>MCh (Pediatric Surgery, Gold Medalist), FISPU, FICS-IS</td>
</tr>
<tr>
<td>Dr. Shabana Naz Shah</td>
<td>PhD. in Pharmaceutical Chemistry</td>
</tr>
<tr>
<td>Raouf Hajji</td>
<td>MD, Specialty Assistant Professor in Internal Medicine</td>
</tr>
<tr>
<td>Vaishnavi V.K Vedam</td>
<td>Master of dental surgery oral pathology</td>
</tr>
<tr>
<td>Surekha Damineni</td>
<td>Ph.D with Post Doctoral in Cancer Genetics</td>
</tr>
<tr>
<td>Tariq Aziz</td>
<td>PhD Biotechnology in Progress</td>
</tr>
</tbody>
</table>
Contents of the Issue

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

1. Entamoeba Coli as a Potent Phagocytic Microorganism. 1-5
2. Prevalence of Multidrug Resistant Tuberculosis and its Associated Factors among Smear Positive TB Patients at Debre Markos Referral Hospital, Northwest Ethiopia. 7-15
3. The Expression of Basal Cytokeratins in Breast Cancers. 17-21
4. Optimization of Citric Acid Production by Substrate Selection using Gamma Ray Induced Mutant Strain of Aspergillusniger. 23-30

v. Fellows
vi. Auxiliary Memberships
vii. Process of Submission of Research Paper
viii. Preferred Author Guidelines
ix. Index
Entamoeba Coli as a Potent Phagocytic Microorganism

By Mosab NM Hamad, Madiha E Elkhairi & Tarig M Elfaki

Elsheikh Abdallah Elbadri University

Abstract- Background: Entamoeba coli is intestinal protozoan amoeba which is regarded tell now as commensal amoeba although their adverse symptoms that they may cause in certain patients.

Objectives:

General Objectives: To know the phagocytic activity of Entamoeba coli against microorganisms.

Specific Objectives: To know the phagocytic activity of Entamoeba coli against microorganisms that inhabit the intestinal tract.

Methodology: The study based on data collected from previous studies.

Result: Entamoeba coli phagocytosed bacterial flora of the gut, fungi of Sphaerita species and even Giardia lamblia trophozoites.

Conclusion: Entamoeba coli is a potent phagocytic microorganism that engulf other microorganisms which may compete it in nutrients.

Keywords: entamoeba coli, phagocytosis, bacteria, parasite, fungi.

GJMR-C Classification: NLMC Code: QW 1

Strictly as per the compliance and regulations of:
Entamoeba Coli as a Potent Phagocytic Microorganism

Mosab NM Hamad, Madiha E Elkhairi & Tarig M Elfaki

Abstract - Background: Entamoeba coli is intestinal protozoan amoeba which is regarded till now as commensal amoebae although their adverse symptoms that they may cause in certain patients.

Objectives:
General Objectives: To know the phagocytic activity of Entamoeba coli against microorganisms.
Specific Objectives: To know the phagocytic activity of Entamoeba coli against microorganisms that inhabit the intestinal tract.

Methodology: The study based on data collected from previous studies.

Result: Entamoeba coli phagocytosed bacterial flora of the gut, fungi of Sphaerita species and even Giardia lamblia trophozoites.

Conclusion: Entamoeba coli is a potent phagocytic microorganism that engulf other microorganisms which may compete it in nutrients.

Keywords: entamoeba coli, phagocytosis, bacteria, parasite, fungi.

I. Introduction

Entamoeba coli are a protozoan endocommensal, inhabiting the lumen of the large intestine of man. There is no reliable evidence that it produces disease in human beings but few workers have reported ingestion of red blood cells by the organism. E. coli was discovered in India by Lewis in 1870 however its detail description was given by Grassi (1879).

a) Geographical Distribution
It is cosmopolitan in distribution and has been stated to occur in about 50% of human population.

b) Life Cycle
Entamoeba coli are a monogenetic organism. Three distinct morphological forms exist airing the life cycle-Trophozoite, Pre-cystic stage and Cystic stage.

Trophozoite of E. coli is about 20 to 30 in diameter with a range from 10 to 50. Trophozoite is unicellular. The cytoplasm is differentiated into outer narrow ectoplasm which is not so prominent and inner granular, vacuolated endoplasm containing bacteria and debris inside food vacuoles. A single nucleus lies inside the endoplasm. The nucleus is a ring like structure with thick nuclear membrane lined with irregularly distributed masses of chromatin and a large, irregular, eccentric karyosome.

Fine linin threads extend between nuclear membrane and karyosome. Trophozoite bears one too many pseudopodia which are short, blunt and granular. Movement is sluggish and usually not directional. The parasite feeds upon bacteria, vegetable cells and other faecal debris present in the large intestine. Dobell (1938) reported that it may ingest R.B.C., occasionally. The trophozoite reproduces by binary fission.
Entamoeba Coli as a Potent Phagocytic Microorganism

Trophozoite changes into spherical uninucleate precystic stage. The precystic stage size ranges from 15 to 45 μm in diameter. It is similar to trophozoite stage, except that it is non-feeding stage and hence food inclusions are not found in the endoplasm. Precystic stage changes into cystic stage.

The cysts are spherical or avoid with size ranging from 10 to 33 μm in diameter. The cyst wall is thick. Immature cyst may have one-two or four nuclei with eccentric karyosome. Occasionally, the cyst may bear 16 or even 32 nuclei. Glycogen vacuoles and chromatid bodies are seen in the endoplasm up to binucleate stage after that they are consumed. Matured cyst is the infective stage. Cysts formed in the large intestine is discharged out of the host's body through faeces. The cysts survive for 3-4 months outside the body of the host and are relatively more resistant to desiccation as compared to those of E. histolytica. The survival rate of the cyst is about 46%.

c) Mode of Infection

Infection to the new host occurs by consuming contaminated food and drinks. The infective stage cysts are carried from faces to the food items through insects and rodents. In the small intestine of the new host excystation occurs during which a single multinucleate amoeba comes out through the cyst wall. Multinucleate amoeba divide into as many immature amoebas as there are nuclei in the cyst. The young amoeba moves down to reach the caecum where they multiply in number and become trophozoites.

d) Pathology

E. coli lives inside the lumen of the large intestine in man. They never enter into the mucosa or sub-mucosa layers or other tissues of the intestine. There is no reliable evidence that it ever produces intestinal lesion, although it has been reported that E. coli occasionally ingest red blood cells.
Entamoeba coli feed on bacterial flora in GIT then it makes disturbance in bacterial flora functions. Entamoeba coli has potent phagocytic activity through which it phagocyted bacterial flora, fungi such as Sapherita species and even other protozoan parasite such as Giardia lamblia trophozoite. (2)

**Phagocytosis**

Is a process by which certain living cells called phagocytes ingest or engulf other cells or particles. The phagocyte may be a free-living one-celled organism, such as an amoeba, or one of the body cells, such as a white blood cell. In some forms of animal life, such as amoebas and sponges, phagocytosis is a means of feeding. In higher animals phagocytosis is chiefly a defensive reaction against infection and invasion of the body by foreign substances (antigens).

**Early Observation**

The presence of foreign particles within cells was first described in the 1860s by pathologist Kranid Slavjansky. In the 1880s Russian-born zoologist and microbiologist Élie Metchnikoff introduced the term phagocyte in reference to immune cells that engulf and destroy foreign bodies such as bacteria. Metchnikoff also recognized that phagocytes play a major role in the immune response, a discovery that earned him a share of the 1908 Nobel Prize for Physiology or Medicine. (3)

Some protozoan parasites can themselves be parasitized. A hyper-parasite! The genus Sphaerita is considered to be a lower fungus and some species are capable of invading the cytoplasm of some amoeboid parasites. Another parasite of parasites is Nucleophaga species which invades the nucleus. Sphaerita, (sometimes called Polyphaga spp.) appear as tightly packed clusters within the cytoplasm and measure approximately 0.5 µm to 1.0 µm.

The parasite show below is possibly an Entamoeba coli, however the nucleus is not visible as it is out of the plane of focus. Sphaerita appears as the small dots within the cytoplasm. (4)
Microscopic examination of a permanently stained fecal preparation revealed the unusual inclusion of a Giardia lamblia cyst within the cytoplasm of an Entamoeba coli trophozoite. (5)

**g) Rationale**
Entamoeba coli tell now regarded as nonpathogenic amoeba although their potent phagocytic activity that enable it to engulf other organisms whom compete it in nutrients and shelter.

**h) Objectives**
*General Objectives:* To know the phagocytic activity of Entamoeba coli against microorganisms.
*Specific Objectives:* To know the phagocytic activity of Entamoeba coli against microorganisms that inhabit the intestinal tract.

**II. MATERIAL AND METHOD**

*Study Design:* Observational study, data collected from previous studies.

**III. RESULTS**
From previous studies we knew that Entamoeba coli engulf certain microorganism and parasitized by others. And that showed potent phagocytic activity of Entamoeba coli.
IV. DISCUSSION

There is adequate agreement with others studies except that said Entamoeba coli is parasitized by Sphaerita species we suggested that Entamoeba coli phagocytosed that Fungal species as a part of their competition in nutrients and shelter.

V. CONCLUSION

Entamoeba coli had a potent phagocytic activity that enable it to engulf other competitive microorganisms.

VI. RECOMMENDATIONS

Another studies are required to know more about that potent phagocytic activity of Entamoeba coli.

ACKNOWLEDGEMENT

Many thanks to all previous researchers that take Entamoeba coli in their eyes, mind and consideration.

REFERENCES RÉFÉRENCES REFERENCIAS

4. http://thunderhouse4-yuri.blogspot.com/2012/05/sphaerita-spp.html
5. https://www.ncbi.nlm.nih.gov/pmc/articles/PMC273646/
This page is intentionally left blank
Prevalence of Multidrug Resistant Tuberculosis and its Associated Factors among Smear Positive TB Patients at Debre Markos Referral Hospital, Northwest Ethiopia

By Teabie Tsega, Dereje Gedle & Tebelay Dilnessa

Debre Markos University

Abstract: Background: The emergence of MDR-TB is a threat for the populations of resource limited countries. In Ethiopia multidrug resistant tuberculosis is becoming a challenge, because of poor adherence to treatment, TB/HIV co-infection, and a few diagnostic and treatment facilities.

Objective: The main aim of this study was to assess prevalence and associated factor for multidrug resistant tuberculosis among smear positive TB patients.

Methods: A retrospective cross-sectional study was conducted among TB patients treated at DOT’s clinic at Debre Markos Referral Hospital from September 1, 2015 to March 10, 2017. Data was enterend and analyzed using SPSS version 20. Logistic regression was employed to assess associated factors with p-value <0.05 as significant.

Results: Of a total of 403 smear positive TB patients 248(61.2%), there was 48(11.9%) drug resistance TB cases. The prevalence of MDR-TB from both new and previously TB treated cases was found to be 1.5%. There was statically significant association between history of previous TB treatment and chance of developing MDR-TB. In this study previously treated patients have 34.26 times more likely to develop MDR-TB than treatment naïve patients [AOR=34.26(95%CI: 4.89-24.11), p=002].

Conclusion: Previous history of TB treatment was found to be significantly associated with MDR-TB.

Keywords: MDR-TB, prevalence, HIV, tuberculosis.

GJMR-C Classification: NLMC Code: WA 400

Strictly as per the compliance and regulations of:

© 2017. Teabie Tsega, Dereje Gedle & Tebelay Dilnessa. 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 of Multidrug Resistant Tuberculosis and its Associated Factors among Smear Positive TB Patients at Debre Markos Referral Hospital, Northwest Ethiopia

Teabie Tsega, Dereje Gedle & Tebelay Dilnessa

Abstract: Background: The emergence of MDR-TB is a threat for the populations of resource limited countries. In Ethiopia multidrug resistant tuberculosis is becoming a challenge, because of poor adherence to treatment, TB/HIV co-infection, and a few diagnostic and treatment facilities.

Objective: The main aim of this study was to assess prevalence and associated factor for multidrug resistant tuberculosis among smear positive TB patients.

Methods: A retrospective cross-sectional study was conducted among TB patients treated at DOT’s clinic at Debre Markos Referral Hospital from September 1, 2015 to March 10, 2017. Data was entered and analyzed using SPSS version 20. Logistic regression was employed to assess associated factors with p-value <0.05 as significant.

Results: Of a total of 403 smear positive TB patients, there were 48 (11.9%) drug resistance TB cases. The prevalence of MDR-TB from both new and previously TB treated cases was found to be 1.5%. There was statically significant association between history of previous TB treatment and chance of developing MDR-TB. In this study previously treated patients have 34.26 times more likely to develop MDR-TB than treatment naïve patients [AOR = 34.26(95% CI: 4.89-24.11), p=002].

Conclusion: Previous history of TB treatment was found to be significantly associated with MDR-TB.

Keywords: MDR-TB, prevalence, HIV, tuberculosis.

1. BACKGROUND

Tuberculosis (TB) is an infectious bacterial disease caused by Mycobacterium tuberculosis, that most commonly affects the lungs. Despite the recent progress of global control efforts, TB remains a major public health burden [1]. In 2014, there were 9.6x10^6 cases and 1.5x10^6 deaths of TB globally [2]. According to WHO Global TB report, Ethiopia is ranked as 15th among 27 high burden MDR-TB countries and 3rd in Africa. The estimated MDR rate was (0.9%–2.8%) for new cases and (5.6%–21%) for retreatment cases [3]. The history of TB treatment has observed sequential development of resistance to anti-TB drugs. Para-amino-salicylic acid and isoniazid were introduced to reduce the development of streptomycin resistance, which heralded the era of combination treatment for TB [4]. Treatment of MDR-TB using second line anti-TB drugs has more adverse events, since provided for an extended period of time (WHO recommendation at least 20 months) and is expensive [5].

Multidrug resistance TB (MDR-TB) is defined as tuberculosis caused by Mycobacterium tuberculosis resistant in vitro to the effects of Isoniazid and Rifampicin with or without resistance to any other drugs [6]. Primary resistance in TB refers to patients infected with M. tuberculosis that is resistant to anti-TB drugs from the outset, prior to anti-TB treatment. MDR-TB is essentially man made that emergence as result of poor TB control including poor supply of management and quality of anti-TB drugs, improper/inadequate treatment which is further fuelled by high prevalence of HIV [7].

MDR-TB is an emerging challenge for TB control programs globally. Emerging and spread of drug resistance TB has encountered as a great challenge in Africa region, Sub-Saharan Africa in particular. Information on the extent of MDR-TB from Africa region is very limited, probably due to poor laboratory facilities, poor surveillance mechanisms and reporting procedures, outdated databases and sub-optimal coverage of infrequent surveys. Sub-Saharan Africa stands the burden of both very high TB incidence and the highest HIV prevalence rates in the world, and represents 14% of the global burden of new MDR-TB cases [8]. Knowledge of the magnitude of MDR-TB is so crucial to allocate resources, and to address prevention and control measures [9]. Therefore, the aim of this study was to assess the prevalence of MDR-TB and associated risk factors at DMRH, Northwest Ethiopia.

II. MATERIALS AND METHODS

a) Study Area and Setting

A facility based cross sectional study was conducted among 403 TB patients who treated and...
registered from September 1, 2015 to March 10, 2017 at Debre Markos Referral Hospital TB clinic. The hospital is a tertiary level hospital that provides health service for inhabitants in East Gojjam Zone and surrounding areas. It provides health service to more than 3.5 million populations in its catchments [10]. In the hospital, DOTS clinic was opened in 2012 under the National Tuberculosis and Leprosy Program of Ethiopia and it gives MDR-TB treatment services.

b) Sample Size and Sampling Technique
All smear positive TB patients registered and treated from September 1, 2015 to March 10, 2017 have been taken as a sample size. The study included 403 smear positive TB patients with full socio-demographic characteristics registered in the TB unit of Debre Markos Referral Hospital. The sample was taken orderly all patients’ recorded and started TB treatment from September 1, 2015- March 10, 2017. Each individual TB patient’s record had been selected from all tuberculosis patients’ records using TB treatment card, TB register form, quarterly report on MDR-TB and case finding format at DMRH.

III. DATA COLLECTION AND ANALYSIS

Data was extracted by reviewing all the necessary registration formats from medical records and treatment charts in TB results at DMRH. The socio-demographic factors together with the clinical profile of the patient were extracted from medical records and treatment charts from the hospital TB database. The collected data were checked manually for the completeness and consistency and the data was cleaned, coded and analyzed using SPSS version 20. Logistic regression was used to determine the association between independent variables and the outcome variable. Odds ratio and 95% confidence intervals were calculated and the result was considered statistically significant at p < 0.05.

a) Ethical Consideration
The study was conducted after it is ethically reviewed by Department of Research and Ethical Review Committee of Debre Markos University. Then supporting letter was written to Debre Markos Referral Hospital. All information during data collection had been confidential; there were no any personal identification which was left on the check list.

b) Operational Definitions
- **MDR-TB:** Is defined as an MDR-TB suspect who is sputum culture positive and whose TB is due to *Mycobacterium TB* that are resistant in-vitro to at least isoniazid and rifampicin
- **New Cases of TB:** Is defined as a newly registered episode of TB or TB treatment for < 1 month
- **Re-treated cases of TB:** A previously treated case is defined as a newly registered episode of TB in a patient with treatment history for TB for 1or more month
- **Primary Resistance:** Patients with TB resistant to one or more anti-TB drugs, but who have never been previously treated for TB
- **Acquired Resistance:** Patients diagnosed with TB who start anti-TB treatment and subsequently acquire resistance to one or more of the drugs used during the treatment
- **Extensive Drug-resistance (XDR):** Resistance to any fluoroquinolone and at least one of the three injectable second line drugs (capreomycin, kanamycin, amikacin)
- **Mono Resistance:** Resistance to only one first line anti TB drug.

IV. RESULTS

a) The Socio-demographic characteristics of smear positive TB patients
A total of 403 smear positive TB patients were enrolled. Of these, 248(61.2%) were males and 155(38.8%) females (Table 1). The prevalence of drug resistance (both RR and MDR-TB patients in this study was 48(11.9%) of which 29(60.4%) were males and 19(39.6%) females. Of all drug resistant TB cases only 6(12.5%) were MDR-TB and the remaining 42(87.5%) were mono-resistance (all rifampicin resistance). From all MDR-TB patients, 4(66.7%) were males and 2(33.3%) were females. Among 403 smear positive TB patients, 6(1.5%) were MDR-TB cases. Of all drug resistance TB cases, 43(89%) of them were rural dwellers and 5(11%) were urban dwellers. On the other hand all patients who were MDR-TB cases were rural dwellers (Table 3).

The highest number of drug resistance TB patients in this study were seen in 2016, 23(47.9%) followed by 22(45.8%) in 2015 and the least observed in 2017, 3(6.3%) (Table 3). Of all drug resistance TB patients across the study period, the age group 16-30 were the most affected which accounts 28(53.2%) followed by age group 31-45, 7(14.6%). The lowest number was observed in the age group 46-60, 3(6.3%). In contrast to this, the majority of MDR-TB patients, 4(66.7%) were in the age group of 0-15 and 16-30 each account 2 and the least were seen in the age group 31-45 and 30-40 which account 1(16.6%) in each group of the total 6 MDR-TB patients. The majority of smear positive TB patients, 355(88.08%) of them were susceptible for rifampicin AFB smear positive TB patients (pulmonary and extra-pulmonary) (Table 4).

b) Prevalence of smear positive TB among patients with age groups in different years
From a total of 25(6.2%) smear positive TB patients found in 0-15 age group, 13(52%) of them were males and the remained, 12(48%) were females. There was an increase amongst males in 2016, 6(46.2%) from the total 25 and amongst females in 2015, 6(46.2%). The
prevalence of smear positive TB patient with the age group of 0-15 was the same in 2015 and 2016, 8 in each year, but there was slightly increasing in 2017, 9 of the total 25 (Table 2).

The highest prevalence of smear positive TB patients in both male and female were observed in the age group 16-30 in 2015 and 2016. From the total number of 236 (58.6%) smear positive TB patients in this age group, 142 (60.1%) males and 94 (49.9%) females. Of these 72 (30.5%) males and 48 (20.3%) females were observed in 2015 followed by 52 (22.0%) males and 33 (24.3%) females in 2016. The least prevalence of smear positive TB patients were seen in 2017, 19 (8.05%) males and 13 (5.5%) females. In addition to this, the prevalence of male patients was relatively higher than that of females across the year 2015-2017 in this age group as indicated in Table 2.

In the age group 31-45, the percentage of smear positive TB patients increased from 2015-2016 and the highest was observed in 2016, 41 (51.3%). The prevalence decreased from 41 (51.3%) in 2016 to 6 (7.5%) in 2017. In addition to this, the percentages of males were relatively higher than that of females across the year from 2015 to 2017. The number of smear positive TB patients registered in this age group during the study period was 80 (19.9%). Fifty one (63.8%) males and 29 (36.2%) females were seen. The second highest prevalence 236 (58.6%) was observed in age group 16-30 (Table 2).

The highest percentage of smear positive TB patients in the age group 46-60 was 23 (50%) registered in 2015 followed by 17 (36.9%) in 2016 and the lowest 6 (13%) in 2017. The percentage of patients was decreasing from 2015, 23 (50%) to 2016, 17 (36.9%). The percentage of male patients was higher than that of female patients across the year from 2015 to 2016, but they were equal in 2017. The total percentage of smear positive TB patients among the age group >60 was 18 (4.5%) registered from 2015 to 2017. Of these, 13 (72.2%) were males and 5 (27.8%) females. The highest percentage was registered in 2015, 9 (55.5%) and the lowest was 3 (16.7%) observed in 2017. The percentage of male patients were relatively higher than that of females across the years from 2015-2017 (Table 2).

c) Drug Resistance Pattern of TB and its Associated Factors

Of all drug resistance TB patients, 39 (81.3%) of them were pulmonary in site and from these 24 (50%) of them were male and 15 (31.3%) of them were females. The rest 9 (19.7%) were extra pulmonary in site, of these 5 (10.4%) were males and 4 (8.3%) females. Of all MDR-TB patients, 4 (66.7%) were belongs to pulmonary in origin and the remaining 2 (33.3%) were extra-pulmonary TB (Figure 1). No statistically significant association was seen between site of TB infection and MDR-TB [COR=0.8 (95% CI 0.74-4.24, p=0.864)]. Forty there were rural dwellers and 5 were urban dwellers among 48 drug resistant TB cases. Therefore, significant association was observed between MDR-TB and residence [AOR=8.2 (95% CI 2.72-14.8), p=0.04] (Table 3).

Of all smear positive TB patients 292 (71.2%), 163 (56.7%) males and 129 (43.3%) females have no previous history of TB treatment. The rest 111 (29.8%), 76 (65.5%) males and 35 (34.5%) females were previously treated for TB. Among drug resistance TB patients the majority of them, 31 (64.6%) were previously treated for TB, of these 18 (58%) were males and 13 (42%) females. Of 48 of drug resistance TB patients 17 (45.4%) were new patients. Eleven (64.7%) were males and 6 (33.4%) females. The highest number of previously treated drug resistance TB patients were seen in 2015, 17 (35.4%) of these 9 (52.9%) were males and 8 (47.1%) females. All MDR-TB patients were previously treated for TB. Of these the majority, 4 (66.7%) of them were under category 4 (failure of new regimen) and the remaining 1 (16.6%) was under category 5 (after failure of retreatment) and the rest 1 (16.6%) was under category 2 (relapse). There was statically significant association between history of previous TB treatment and the chance of developing MDR-TB [AOR=34.26 (95% CI: 4.89-24.11), p=0.002] (Table 3).

d) Prevalence of Drug Resistance TB in HIV positive patients

The prevalence of TB/HIV co-infection was 29 (7.2%). Of these, 17 (4.16%) were males and 12 (2.9%) females. The majority of patients, 374 (92.8%) were HIV negative. Age group 16-30 years, 19 (4.67%) took the major share followed by 31-45 years, 9 (2.19%) and the least affected age group was 46-60 and >60 each account 0% in retroviral infection among smear positive TB patients. Males were the most affected group in TB/HIV co-infection (Table 2).

The prevalence of drug resistance TB and HIV co-infection in this study was 12 (24.9%). Among these 9 (18.6%) were males and 3 (6.3%) females. Across the study period males were predominate over females in drug resistance TB and HIV co-infection except in 2015, in this case both sexes were equal in number. On the contrary, none of MDR-TB patients were HIV positive. The highest number of drug resistance TB and HIV co-infection were seen in 2016, 8 (12.5%) and the least (0%) were seen in 2017. There was no statistical significant association between drug resistant TB and HIV status [COR=22.5 (95% CI 0.35-98.5, p=0.998)] (Table 3).

e) Trends of MDR- TB across the study period

From the total of 48 drug resistance TB patients, 42 (87%) of them were RR (rifampicin resistance), of these 25 (59.5%) were males and 17 (40.5%) females. Of all drug resistant, 6 (12.5%) of them were MDR-TB patients, 4 (66.7%) males and 2 (33.3%) females. When observing the trends of MDR-TB across the study period
among smear positive TB patients, the number of MDR-TB patients were 3(0.74%), 2(0.49) and 1(0.24%) in 2015, 2016 and 2017 respectively. So the trends of MDR-TB was decreasing from 3(0.74%) in 2015 to 1(0.24%) in 2017 (Table 3).

V. Discussion

The prevalence of MDR-TB from both new and previously TB treated cases in this study was found to be 1.5%. The finding in this study was lower than previous study in the same study area [11]. Multidrug resistance TB is estimated to be 3.7% of newly diagnosed patients with TB and 20% of previously treated patients around the world as shown by WHO 2012 report [8]. On the other hand, in the fourth WHO global report on anti-TB drug resistance in the world, data are reported from eight countries of the Region, and MDR-TB rate in this region were 2% among new cases, 35.3% among previously treated cases and 5.4% from all or combined cases [6]. In a previous study conducted from January 2011 to December 2013 stated that from a total of 2149 TB patients received in-patient treatment at St. Peter TB specialized referral hospital, 780(38%) patients were MDR-TB (culture positive) which is much higher than the result of this study [12].

A study finding in Northeastern China showed the prevalence of MDR-TB of 8.7% [13]. Similar study findings in New Delhi, India, shows from sputum positive pulmonary TB clients enrolled, the prevalence of MDR-TB among newly diagnosed pulmonary TB patients was 1.1% [14]. Another study in India on the pulmonary TB drug resistant shows 8% MDR-TB [15]. In contrast, all the above mentioned results were higher than the result found in this study and all MDR-TB patients were previously treated for tuberculosis, but there were MDR-TB cases in treatment naive patients.

A research conducted in Dessie town, among 434 TB cases of TB treatment, 9(2.1%) were found to be MDR-TB cases which is a bit higher than this study finding [16]. On the other hand, a study which was conducted in Addis Ababa, at St TB specialized hospital from January 2011 to December 2013, a total of 2149 TB patients were received in-patient TB treatment in this hospital, of which 780(38%) patients were MDR-TB [12]. This higher prevalence of MDR-TB might be due to most of the patients were referral cases. Other studies conducted in the same study area at Debre Markos referral hospital showed, the prevalence of MDR-TB was 2.3% and 3% which is higher than the current study [11, 17]. But most MDR-TB cases were observed in males, 16-30 age groups and rural dwellers in agreement to the current study.

The result is slightly higher than the result of this study. But gender distributions of MDR-TB patients were almost similar to the previous studies. Regarding the trends of MDR-TB in the study area, previous study shows an increasing trend of MDR-TB patients across the study period which was in contrast to this study. The trend of MDR-TB was 0%, 0.3%, 0.6%, 0.5% and 0.9% for 2011, 2012, 2013, 2014, and 2015 respectively. The trend of MDR was increasing in the study area from 0.0% in 2011 to 0.9% in 2015 [18-22]. The decrease in the prevalence of MDR TB in the current study area may be due to better information of the community about the cause, transmission, prevention and treatment of tuberculosis. And commitment of the health professionals to strictly follow TB patients during the intensive and continuation phase of TB treatment. The new cases MDR-TB prevalence of this study was found to be null (0%) from all smear positive TB cases, which was extremely lower compared to other studies mentioned above. The possible reason for this low figure finding could it be low MDR-TB detection status of the hospital. On the other hand, the higher prevalence of MDR-TB in previously TB treated patients may be due to a poor adherence of patients to anti TB drugs by different reason.

There was no statistically significant association between age groups and MDR-TB occurrence from this study, which is similar in a study finding in Dessie administration [16]. In contrast to this, age was considered a risk factor for MDR TB as it was explained in a previous study, Ethiopia [23]. Age group at 25-44 years in Bangladesh was a risks factor of MDR-TB. Sex was not significantly associated with MDR-TB according to this study finding. Similarly, there was no statistically significant association between sex and MDR-TB occurrence in a study conducted Dessie city administration in Ethiopia [16]. In contrast to this a nationwide survey conducted in China showed that, female gender were a risk factors for MDR-TB [13]. But a study in Nigeria showed gender was not significantly associated with MDR-TB [24]. Another study finding in Thailand also showed male gender as risk factors for MDR-TB [25]. In Ethiopia, male gender was a risk factor for MDR-TB in previous study [26] which is in contrast to current study.

Regarding treatment status of MDR-TB patients, all 6(100%) of them were previously treated for TB. And there was statically significant association between history of previous TB treatment and the chance of developing MDR-TB. In this study previously treated for TB patients have 34.26 times more risk to develop MDR-TB than treatment naïve patients. This result is similar to the report in a previously conducted study in Dessie City administration, Ethiopia stated that prevalence rate of MDR-TB from new TB cases, retreated cases and combined of all were found to be 0.3/100, 21.6/100 and 2.1/100 respectively from all forms of TB cases. The prevalence rate of acquired MDR-TB was similar to the combined prevalence rate above since all MDR-TB...
cases were acquired whereas, primary MDR-TB rate was null [16].

The prevalence of MDR-TB in previous study was 2.3% of which 0.2% new cases and 2.1% previously treated cases. Drug resistance was strongly associated with previous treatment [27, 28]. This is comparable with the result of this study. In this study, there was no statically significant association between occurrences of MDR-TB and HIV status of patients. This result is similar to the finding in Kenya, Malawi, Tanzania, Cote d’Ivoire and France [29-32]. The Global Project of MDR-TB, which has been gathering data since 1994 from 7 countries, none of which have a high prevalence of HIV infection and there was no association between HIV infection and MDR-TB in 5 of these countries, where as a significant association was observed between MDR-TB and HIV infection in 2 countries Latvia and Ukraine [33, 34]. HIV was a risk factor for TB/MDR-TB accordingly to, WHO report at California; US during 2011 which shows HIV contribute 4.5% MDR-TB cases [8].

With respect to the contribution of site of TB to multidrug resistant in the current showed that from all MDR-TB cases 4(4/6) of them were from pulmonary TB type and the rest 2 (2/6) were extra-pulmonary. A comparable result was noted in another study in Ethiopia which showed pulmonary TB type was a risk factor for MDR-TB [26]. In contrast to the result of this study, a study in southern Ethiopia showed that HIV have statistically significant association for both acquired MDR-TB and primary MDR-TB [27].

VI. Conclusion

Prevalence of MDR-TB for both new and retreated TB cases from all smears positive TB patients at DMRH were found to be 1.5%. Previous history of TB treatment was found to be significantly associated with MDR-TB. In this study, age, sex and HIV status were not associated with MDR-TB. Counseling related to anti-TB drugs adherence during intensive and continuation phase of TB treatment is mandatory to decrease MDR-TB. Further prospective study is necessary to have more information about MDR-TB in the country in general and in study area in particular.

a) Limitation of the Study

The data was collected by using secondary data source from already recorded documents, so there were some difficulties in getting all the necessary information regarding the study across the study period. Even thought, the hospital started to give MDR TB diagnosis and treatment in February, 2014, the study included data only for 3 years (2015-2017) due to time constraint.

b) Abbreviations


c) Ethical approval and consent to participate

The study was approved by research and ethical review committee of Debre Markos University. All information during data collection was confidential; there was not any personal identification which was left on the check list.

d) Consent for Publication

Not Applicable.

e) Availability of data and materials

All data generated and analyzed during this study were included in the manuscript.

f) Competing Interests

Authors declare that they have no competing interests.

g) Funding

No funding source.

Acknowledgements

We would like to acknowledge administrative and TB/HIV treatment and control center staff of Debre Markos Referral Hospital.

References Références Referencias

4. WHO. Shorter treatment regimens for multidrug-resistant tuberculosis, treatment outcomes observed in Bangladesh for Multidrug-resistant-TB cases treated with a nine months regimen. 2013; 1-2. Available at: www.who.int/tb/challenges/mdr

**Table 1:** The socio-demographic characteristics of smear positive TB patients by age group, sex and retrovirus infection status at DMRH, Northwest Ethiopia, March 2017, (N=403)

<table>
<thead>
<tr>
<th>Age group</th>
<th>Sex</th>
<th>HIV Status</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>M</td>
<td>F</td>
</tr>
<tr>
<td>0-15</td>
<td>13</td>
<td>11</td>
</tr>
<tr>
<td>16-30</td>
<td>142</td>
<td>92</td>
</tr>
<tr>
<td>31-45</td>
<td>51</td>
<td>29</td>
</tr>
<tr>
<td>46-60</td>
<td>29</td>
<td>17</td>
</tr>
<tr>
<td>&gt;60</td>
<td>13</td>
<td>6</td>
</tr>
<tr>
<td>Total</td>
<td>248</td>
<td>155</td>
</tr>
</tbody>
</table>

**Table 2:** The prevalence of smear positive TB patients among age groups and HIV status in different years at DMRH, Northwest Ethiopia

<table>
<thead>
<tr>
<th>Year</th>
<th>Age group</th>
<th>Sex</th>
<th>%</th>
<th>Total</th>
<th>HIV Status</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>M</td>
<td>F</td>
<td>%</td>
<td>Total</td>
</tr>
<tr>
<td>2015</td>
<td>0-15</td>
<td>2</td>
<td>25</td>
<td>6</td>
<td>75</td>
</tr>
<tr>
<td></td>
<td>16-30</td>
<td>71</td>
<td>59.7</td>
<td>48</td>
<td>40.3</td>
</tr>
<tr>
<td></td>
<td>31-45</td>
<td>19</td>
<td>57.6</td>
<td>14</td>
<td>42.4</td>
</tr>
<tr>
<td></td>
<td>46-60</td>
<td>14</td>
<td>60.9</td>
<td>9</td>
<td>39.1</td>
</tr>
<tr>
<td></td>
<td>&gt;60</td>
<td>8</td>
<td>80</td>
<td>2</td>
<td>20</td>
</tr>
<tr>
<td></td>
<td>Total</td>
<td>114</td>
<td>59.1</td>
<td>79</td>
<td>40.9</td>
</tr>
<tr>
<td>2016</td>
<td>0-15</td>
<td>6</td>
<td>75</td>
<td>2</td>
<td>25</td>
</tr>
<tr>
<td></td>
<td>16-30</td>
<td>52</td>
<td>61.2</td>
<td>33</td>
<td>38.86</td>
</tr>
<tr>
<td></td>
<td>31-45</td>
<td>28</td>
<td>62.3</td>
<td>13</td>
<td>37.7</td>
</tr>
<tr>
<td></td>
<td>46-60</td>
<td>12</td>
<td>70.6</td>
<td>5</td>
<td>29.4</td>
</tr>
<tr>
<td></td>
<td>&gt;60</td>
<td>3</td>
<td>60</td>
<td>2</td>
<td>40</td>
</tr>
<tr>
<td></td>
<td>Total</td>
<td>101</td>
<td>64.7</td>
<td>55</td>
<td>34.3</td>
</tr>
<tr>
<td>2017</td>
<td>0-15</td>
<td>5</td>
<td>55.6</td>
<td>4</td>
<td>44.4s</td>
</tr>
<tr>
<td></td>
<td>16-30</td>
<td>19</td>
<td>59.4</td>
<td>13</td>
<td>40.6</td>
</tr>
<tr>
<td></td>
<td>31-45</td>
<td>4</td>
<td>66.7</td>
<td>2</td>
<td>33.3</td>
</tr>
<tr>
<td></td>
<td>46-60</td>
<td>3</td>
<td>50</td>
<td>3</td>
<td>50</td>
</tr>
<tr>
<td></td>
<td>&gt;60</td>
<td>2</td>
<td>75</td>
<td>1</td>
<td>25</td>
</tr>
<tr>
<td></td>
<td>Total</td>
<td>33</td>
<td>58.9</td>
<td>23</td>
<td>41.1</td>
</tr>
</tbody>
</table>
Table 3: Resistance pattern and treatment categories of MDR-TB patients at DMRH Northwest Ethiopia from September 2015-March 2017

<table>
<thead>
<tr>
<th>Year</th>
<th>Treatment Status</th>
<th>Resistance Type</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>New</td>
<td>Previously Treated</td>
</tr>
<tr>
<td></td>
<td>M %</td>
<td>F %</td>
</tr>
<tr>
<td>2015</td>
<td>4</td>
<td>80</td>
</tr>
<tr>
<td>2016</td>
<td>6</td>
<td>60</td>
</tr>
<tr>
<td>2017</td>
<td>1</td>
<td>50</td>
</tr>
<tr>
<td>Total</td>
<td>11</td>
<td>64.7</td>
</tr>
</tbody>
</table>

RR- Rifampicin resistance, MDR- Multidrug resistance

Table 4: Analysis of socio-demographic and clinical factors for MDR-TB patients at DMRH, Northwest, Ethiopia, March 2017

<table>
<thead>
<tr>
<th>Variables</th>
<th>Resistant Pattern</th>
<th>COR (95% CI)</th>
<th>P-value</th>
<th>AOR (95% CI)</th>
<th>P-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sex</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>M</td>
<td>29</td>
<td>219</td>
<td>1.6(0.06-9.11)</td>
<td>0.574</td>
<td>-</td>
</tr>
<tr>
<td>F</td>
<td>19</td>
<td>136</td>
<td>1</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Age</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>0-15</td>
<td>6</td>
<td>28</td>
<td>1</td>
<td></td>
<td></td>
</tr>
<tr>
<td>16-30</td>
<td>28</td>
<td>206</td>
<td>0.73(0.02-0.86)</td>
<td>0.340</td>
<td>-</td>
</tr>
<tr>
<td>31-45</td>
<td>7</td>
<td>73</td>
<td>0.33(0.21-5.3)</td>
<td>0.997</td>
<td>-</td>
</tr>
<tr>
<td>46-60</td>
<td>3</td>
<td>43</td>
<td>0.86(0.81-7.2)</td>
<td>0.997</td>
<td>-</td>
</tr>
<tr>
<td>&gt;60</td>
<td>4</td>
<td>15</td>
<td>0.41(0.24-10.29)</td>
<td>0.768</td>
<td>-</td>
</tr>
<tr>
<td>Previous history of TB</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Yes</td>
<td>31</td>
<td>80</td>
<td>31.0(9.78-74.44)</td>
<td>0.001</td>
<td>34.26(4.89-24.11)</td>
</tr>
<tr>
<td>No</td>
<td>17</td>
<td>275</td>
<td>1</td>
<td></td>
<td>1</td>
</tr>
<tr>
<td>HIV status</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Positive</td>
<td>12</td>
<td>17</td>
<td>22.5(0.35-98.5)</td>
<td>0.998</td>
<td>-</td>
</tr>
<tr>
<td>Negative</td>
<td>36</td>
<td>338</td>
<td>1</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Residence</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Rural</td>
<td>43</td>
<td>200</td>
<td>5.4(1.64-10.63)</td>
<td>0.025</td>
<td>8.2(2.72-14.8)</td>
</tr>
<tr>
<td>Urban</td>
<td>5</td>
<td>108</td>
<td>1</td>
<td></td>
<td>1</td>
</tr>
<tr>
<td>Site of TB</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>PTB</td>
<td>39</td>
<td>238</td>
<td>0.8(0.74-4.24)</td>
<td>0.864</td>
<td>-</td>
</tr>
<tr>
<td>EPTB</td>
<td>9</td>
<td>70</td>
<td>1</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

R=Resistant, S=Sensitive, PTB=Pulmonary Tuberculosis, EPTB=Extra-pulmonary Tuberculosis
Figure 1: Site of drug resistance TB among patients at Debre Markos Referral Hospital, Northwest Ethiopia, March 2017
This page is intentionally left blank
The Expression of Basal Cytokeratins in Breast Cancers

By Dr. Vidhya Lakshmi S & Dr. Seyed Rabia

PSG Institute of Medical Sciences & Research

**Abstract-** Introduction: Treatment for breast cancer is based on the expression of the immunomarkers such as ER, PR and HER2/neu. Cases which are negative to all the three immunomarkers, are called Triple Negative Breast Cancers (TNBC) and they have a poor prognosis. Recent studies have shown that some of the TNBCs express cytokeratins CK 5/6 (subcategorizing them as basal-like breast cancers) and these respond well to anthracycline-based chemotherapy.

**Aim and Objectives:** To study the expression of basal cytokeratins CK 5/6 in breast carcinomas reported in our centre and to correlate with histological type, grade, size, clinical features and ER, PR and HER2/neu status.

**Methods:** Tissues of 44 cases of breast carcinoma diagnosed between June 2009 and May 2014 were retrieved. Immunohistochemical staining for CK 5/6 was done and it was correlated with parameters such as histopathological type, grade, size, invasion and ER, PR and HER2/neu status.

**Keywords:** triple negative breast cancers, cytokeratin 5/6, basal-like breast carcinoma.

**GJMR-C Classification:** NLMC Code: QU 475

© 2017. Dr. Vidhya Lakshmi S & Dr. Seyed Rabia. 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 Expression of Basal Cytokeratins in Breast Cancers

Dr. Vidhya Lakshmi S \textsuperscript{a} & Dr. Seyed Rabiɑ\textsuperscript{a}

\textbf{Abstract- Introduction:} Treatment for breast cancer is based on the expression of the immunomarkers such as ER, PR and HER2/neu. Cases which are negative to all the three immunomarkers, are called Triple Negative Breast Cancers (TNBC) and they have a poor prognosis. Recent studies have shown that some of the TNBCs express cytokeratins CK 5/6 (subcategorizing them as basal-like breast cancers) and these respond well to anthracycline-based chemotherapy.

\textbf{Aim and Objectives:} To study the expression of basal cytokeratins CK 5/6 in breast carcinomas reported in our centre and to correlate with histological type, grade, size, clinical features and ER, PR and HER2/neu status.

\textbf{Methods:} Tissues of 44 cases of breast carcinoma diagnosed between June 2009 and May 2014 were retrieved. Immunohistochemical staining for CK 5/6 was done and it was correlated with parameters such as histopathological type, grade, size, invasion and ER, PR and HER2/neu status.

\textbf{Results:} Eight of the breast carcinomas (18\%) were categorized as Triple Negative Breast Cancers (TNBC) as they were negative for ER, PR and HER2/neu. Four of the TNBCs (50\%), were positive for CK 5/6. Significant statistical correlation was observed between the size of the tumour and positive CK 5/6 expression. All CK 5/6 positive cases were of high grade.

\textbf{Conclusion:} The routine use of CK 5/6 is recommended in all cases of TNBCs, as 50\% of them are positive for these markers. Patients in this subcategory could benefit from anthracycline-based chemotherapy.

\textbf{Keywords:} triple negative breast cancers, cytokeratin 5/6, basal-like breast carcinoma.

\section{I. Introduction}

Breast cancers are a diverse group of diseases that vary remarkably in terms of clinical presentation, histology, behavior and genetic characteristics [1]. There has been a steady increase in the incidence of breast cancers worldwide and especially in the developing countries, mainly attributable to globalization causing adaptation of western lifestyle and improved access to diagnostic modalities. As per the International agency for research on cancer, the number of new cases of female breast cancers in India in the year 2012 was 144,937. [2] The mortality rate in the Indian cohort was 50\% compared to that in USA, where only one woman out 5-6 patients die of breast cancer.

Breast cancers that express Estrogen and Progesterone receptors can be treated by hormonal manipulation [3]. Targeted therapy towards HER2 neu has great success and Trastuzumab has been introduced as an adjuvant drug in those showing over expression of Her 2 neu [4]. A subset of breast cancers have been found to show no expression of any of the above mentioned markers. These have been labelled as Triple Negative Breast Cancers (TNBCs). Though hormonal manipulation is of no use in this subset, they have found to show expression of other markers such as basal Cytokeratins and EGFR. They have greater sensitivity to anthracycline based chemotherapy despite poor pathologic complete response [5].

This study focuses on identifying the cases of breast cancer at our centre, performing immunohistochemical studies of the basal Cytokeratin CK5/6 in them and studying their expression and correlation with various clinicopathological parameters.

\section{II. Materials and Methods}

Cases of breast carcinomas diagnosed between the years 2009 and 2014 were included in our study. The study was performed after getting approved by the Institutional Human Ethics Committee (IHEC). A few of the cases were rejected owing to the absence of sufficient clinical information, ER/PR studies or if blocks were unavailable. The requisition form sent by the operating surgeon was used for deriving information such as age, site, nodal status and other gross findings. Hematoxylin and eosin stained slides from representative sections of the breast tumours were used for grading and assessing the histological type of tumour, evidence of lymphovascular invasion, perineural invasion and skin involvement. Immunohistochemical staining for Estrogen Receptor, Progesterone Receptor, Her2neu and CK5/6 was performed on these sections after antigen retrieval in pressure cooker followed by EDTA buffer at an alkaline pH (pH of 9).

The antibody reagent clones were Clone EP1 by DAKO, Clone PgR 636 by DAKO, Anti-v-erbB-2 Clone CB11 by Biogenix and FLEX Monoclonal Mouse Anti-human Cytokeratin5/6 (Clone D5/16B4) for ER, PR, Her2neu and CK5/6 respectively. A two stage process involving binding of primary antibody to the targeted epitope; second step by identifying a secondary antibody bound to a dextran polymer with the help of horseradish peroxidase enzyme attached to a chromogen.

\textsuperscript{a}Author a: e-mail: drvidhylakshmi@yahoo.co.in

© 2017 Global Journals Inc. (US)
The various parameters analyzed were age, histological type, size of the tumour, grade, skin, lymphovascular and perineural invasions, number of axillary lymph nodes showing metastasis and staining properties of ER, PR, Her2neu and CK5/6.

Based on studies conducted by Rakha et al [1], Laakso et al [6] an arbitrary scoring system was drawn up for quantifying the expression of CK5/6. (Table: 1)

<table>
<thead>
<tr>
<th>Score</th>
<th>Description</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>Less than 1 % positivity</td>
</tr>
<tr>
<td>1 +</td>
<td>1-10% tumour cells are positive</td>
</tr>
<tr>
<td>2 +</td>
<td>10-50% tumour cells are positive</td>
</tr>
<tr>
<td>3 +</td>
<td>More than 50% tumour cells are positive</td>
</tr>
</tbody>
</table>

III. RESULTS

44 cases of female breast cancer were under study. The ages ranged between 33 and 67 years. The age group that had the most number of cases was 41 to 50 years. The commonest histological grade in our study was Grade 2, with 24 cases and 9 cases were grade 3(Fig:1).

Fig. 1: Invasive ductal carcinoma NOS, H&E 400x

88% of the tumours were of the infiltrating ductal carcinoma, not otherwise specified (NOS). Two cases of micro papillary carcinoma, a case of metaplastic carcinoma and papillary carcinoma were included. Most cases (22) were of sizes between 2cm-5 cm. Lympho vascular invasion was seen in 20 out of 44 cases, with most cases belonging to Grade 2. Perineural invasion (Fig: 2) was seen in only two cases.

Fig. 2: Invasive ductal carcinoma with perineural invasion, 400x

Estrogen receptor expression was seen in around 45% of cases and 52% of the cases showed Her 2 neu over expression. (Fig 3)

Fig. 3: Histogram showing distribution of ER/Her 2 neu & grade

Size of the tumour statistically correlated with CK 5/6 positivity. Larger tumours had a greater incidence of CK 5/6 positivity with the largest tumour being 13.5 cm in size. (Fig 4)
Correlation between CK 5/6 with invasive and prognostic features was performed and we observed that the tumors with higher grade, lymphovascular, perineural invasion and extensive lymph node metastases showed greater CK5/6 expression than their respective counterparts. (Fig 5)

IV. Discussion

Breast carcinomas have emerged as the most common malignancy in Indian women. Not only is their incidence high, but the fatality rate of these cases exceeds those of the western population [6]. The cure rates, quality and length of life have improved in these women after the development of targeted therapy. Glass et al [7] observed quantitative and qualitative trends in breast cancer incidence. There has been a tremendous increase, particularly in ER positive tumours. The reason for the exponential rise has been attributed to the use of post-menopausal hormone replacement therapy and widespread utilization of mammography.

Breast cancers have been sub classified into 4 molecular subtypes.
1. Luminal A – High expression of luminal cytokeratins and hormone receptors. ER/PR-positive, HER2/neu-negative.
2. Luminal B - Expression of luminal cytokeratins are seen. ER/PR-positive, HER2/neu expression-variably positive. Higher grade and proliferation than Luminal A.
3. HER2/neu - Low expression of ER. High expression of HER2/neu and 17q12. ER/PR negative. HER2/neu positive. High grade, TP53 mutations present and higher likelihood of nodal metastasis.

Treatment response to endocrine therapy is good in ‘Luminal type A’ tumours. The response in ‘Luminal B’ tumours is not as satisfactory as in Luminal A tumours. Her2 neu class of tumours responds to Trastuzumab. The basal like tumours do not respond to endocrine therapy or Trastuzumab.

The subsets of breast carcinoma which are not susceptible to conventional therapy, have a paradigm shift in molecular genetics and immunohistochemical expression. These are the Basal-like breast carcinomas and the Triple Negative Breast Cancers.

The overall percentage of ER positive cases in our study was 45%, lesser when compared with western literature. (Fig 6). This is consistent with findings in a study conducted by Ambroise et al [8], which concluded saying that hormonal expression is lesser in the south Indian population. We noted that 54% of ER positive tumours were node positive and most ER positive neoplasms [9] were less than 2 cm in size.
We inferred that due to the large size of the tumours in our study, there was increased nodal metastasis. Almost all the women with ER positive cases belonged to the 35 to 65 year age group.

Normalization technique was introduced for standardization of results and to avoid discordance between immunohistochemistry and FISH results. There is improved accuracy of HER2 studies using a subtraction scoring system in which a signal score of non-neoplastic breast epithelium is subtracted from that of the tumour [10]. Using this system, the proportion of HER2 positive tumours in our study is 63%. (Fig 7). This stresses the need to look into other markers and their routine use in South Indian cohorts.

Dolle et al [11] inferred that Triple Negative Breast Cancers (TNBCs) are breast cancer subtypes associated with high mortality rate and resistance to hormonal manipulation and Herceptin. Since these tumours are negative for ER, PR and HER2, newer markers are to be identified for this subtype. These tumours have been seen with increased incidence in younger women (aged 45 years or younger). Our study showed close correlation, with the mean age of women with TNBCs being 46 years and nearly a third of the women were 40 or younger.

The purpose of our study was to identify a newer basal marker and observe the expression and clinic-pathological in cases of breast cancer at our centre. The basal marker that we selected for our study was CK 5/6[12]. Clark et al suggested that CK5 is positive in breast progenitor cells, which are believed to be the cell of origin in basal-like breast cancers (Rakha) [1]. In our study, 25% of the cases were basal-like, with all of these tumours falling into either Grade 2 or Grade 3. Thus CK 5/6 was positive in 50 % of TNBCs (Fig: 8). The only case of metaplastic tumour was negative for basal markers.

Nielson et al [13] observed that a panel consisting of ER, HER2 and CK 5/6 to identify the basal-like subset was useful as this immunohistochemical combination had a 76% sensitivity and 100% specificity rate when compared with genetic microarray analysis. The conclusions drawn from our study is that CK5/6 positivity was seen in tumours of larger size and higher grades.
ACKNOWLEDGEMENT

The skilled team of technicians at our histopathology laboratory who helped us with the practical aspects of this study.

REFERENCES Références Referencias

This page is intentionally left blank
Optimization of Citric Acid Production by Substrate Selection using Gamma Ray Induced Mutant Strain of *Aspergillus Niger*

By Shamima Nasrin, Mesbah Uddin Ansary & Md. Khorshed Alam

*Jahangirnagar University*

**Abstract:** The worldwide demand for citric acid is increasing with the rising population and industrialization. The growing demand for citric acid and the need for alternative materials as substrates in the recent years have led to the choice of a novel and economically viable substrate, namely jackfruit (outer portion) and molasses for citric acid biosynthesis. Hydrolysis these substrates with 0.05N HCl followed by fermentation using two isolates of *Aspergillus niger* were investigated for citric acid production under submerged culture condition in a period of 15 days. The products of the microbial metabolism namely residual sugar, total titratable acidity (TTA), citric acid, and biomass contents were determined periodically. Maximum citric acid production was found after 12 days of fermentation for both isolates, namely *Aspergillus niger* CA16, the parent strain and gamma ray induced mutant *Aspergillus niger* 79/20. Citric acid production was found highest in the absence of Prescott salt by *Aspergillus niger* CA16 in mixed fermentation medium which was about 16.35 mg/ml and lowest in jackfruit medium, 12.88 mg/ml at day 12. Whereas in the presence of Prescott salt, lowest citric acid production was also found in jackfruit medium, 7.21 mg/ml and highest in mixed medium, 11.54 mg/ml. In case of the previously isolated gamma-ray induced mutant *Aspergillus niger* 79/20, the yield seems to be higher under similar experimental condition.

**Keywords:** *aspergillus niger* 79/20, citric acid, titratable acidity.

**GJMR-C Classification:** NLMC Code: QW 4

© 2017, Shamima Nasrin, Mesbah Uddin Ansary & Md. Khorshed Alam. 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.
Optimization of Citric Acid Production by Substrate Selection using Gamma Ray Induced Mutant Strain of Aspergillus niger

Shamima Nasrin a, Mesbah Uddin Ansary a & Md. Khorshed Alam b

Abstract: The worldwide demand for citric acid is increasing with the rising population and industrialization. The growing demand for citric acid and the need for alternative materials as substrates in the recent years have led to the choice of a novel and economically viable substrate, namely jackfruit (outer portion) and molasses for citric acid biosynthesis. Hydrolysis of these substrates with 0.05N HCl followed by fermentation using two isolates of Aspergillus niger were investigated for citric acid production under submerged culture condition in a period of 15 days. The products of the microbial metabolism obtained in case of the parent strain.

Abstract: The products of the microbial metabolism obtained in case of the parent strain.

worldwide demand for citric acid is met by fermentation mainly by the process involving the filamentous fungus A. niger. A number of carbon sources may be used for citric acid fermentation. For commercial reasons, the uses of molasses, sucrose or glucose syrups are favored. The use of molasses in particular is desirable because of its low cost availability.

A. niger is capable of producing very high levels of citric acid, about 90% of the theoretical yield from a carbohydrate source. For an efficient citric acid production, the growth of Aspergillus in pellet form is desirable and this can be achieved by process optimization. There is a great worldwide demand for citric acid consumption due to its low toxicity compared with other acidulants used mainly in the pharmaceutical and food industries. Global production of citric acid has now reached 1.4 million tones and there is annual growth of 3.5–4.0 % in demand/consumption. A high rate of acidogenesis in A. niger is observed only under conditions of high glycolytic metabolism and can be induced by the addition of an excess amount of sucrose or other carbohydrates which induce a high rate of glycolytic catabolism. In this production technique, which is still the major industrial route to citric acid used today, cultures of Aspergillus niger are fed on a sucrose or glucose-containing medium to produce citric acid. The source of sugar is corn steep liquor, molasses, hydrolyzed corn starch or other inexpensive sugary solutions. Bangladesh, at present, imported cent percent citric acid from foreign countries. High production depends to a great extent on the strain used and its response to the composition of the medium can show a great deal of variability. Industrial production of this chemical by fermentation using cheap raw materials is helpful in economic development of our country. Keeping in view the future requirements and also the availability of cheap raw material, efforts were made to develop the process for citric acid fermentation, based on our local resources such as molasses from sugar mills and outer portion of jackfruit. So the purpose of present study describes the feasibility of using raw and cheap materials such as molasses and outer portion of jackfruit for citric acid fermentation and to use parent strain CA16 & gamma-ray induced mutants for high citric acid yielding strain 79/20 of Aspergillus niger.

I. Introduction

Citric acid is one of the world’s largest tonnages of fermentation products. It is widely used in the food beverage industries as an acidifying and flavor-enhancing agent, pharmaceutical, chemical, cosmetic and other industries for applications such as acidulation, antioxidation, flavor enhancement, preservation, plasticizer and as a synergistic agent. The sources of sugar are corn steep liquor, molasses, hydrolyzed corn starch or other inexpensive sugary solutions. Bangladesh, at present, imported cent percent citric acid from foreign countries. High production depends to a great extent on the strain used and its response to the composition of the medium can show a great deal of variability. Industrial production of this chemical by fermentation using cheap raw materials is helpful in economic development of our country. Keeping in view the future requirements and also the availability of cheap raw material, efforts were made to develop the process for citric acid fermentation, based on our local resources such as molasses from sugar mills and outer portion of jackfruit. So the purpose of present study describes the feasibility of using raw and cheap materials such as molasses and outer portion of jackfruit for citric acid fermentation and to use parent strain CA16 & gamma-ray induced mutants for high citric acid yielding strain 79/20 of Aspergillus niger.
Aspergillus niger is a haploid filamentous fungus and is a very essential microorganism in the field of biology. A. niger is cultured for the industrial production of many substances. Various strains of A. niger are used in the industrial preparation of citric acid (E330) and gluconic acid (E574) and have been assessed as acceptable for daily intake by the World Health Organization. A. niger is important because of its involvement in producing citric acid as well as industrial enzymes, such as amylases, proteases, and lipases. The uses of these enzymes are essential because of its importance for transformation to food enzymes. For example, A. niger glucoamylase is used in the production of high fructose corn syrup, and pectinases are used in cider and wine clarification. Glucose oxidase is used in the design of glucose biosensors, due to its high affinity for D-glucose. A variety of carbohydrate sources such as beet molasses, cane molasses, sucrose, commercial glucose, starch hydrolysates etc., have been used for citric acid production. Among these, sucrose, cane and beet molasses have been found to be the best choice (Kapoor et al., 1982).

II. Materials and Methods

This study was done in the research laboratory of the Department of Biochemistry and Molecular Miology at Jahangirnagar University and at Institute of Food and Radiation Biology, Atomic Energy Research Establishment, Bangladesh during July 2009 and June 2010.

Parent strain Aspergillus niger CA16 and mutant strain Aspergillus niger 79/20 were first grown on agar slant medium. Each of the properly processed substrates [Molasses, jackfruit (outer potion) and mixed substrates] was hydrolyzed by 0.05 N HCl and filtered which were then used as medium for submerged fermentation. Each substrate were divided into two groups and were fermented separately, one in the presence of Prescott salt and the other in the absence. Each of the groups of the three types of media were further divided into another two subgroups and one of them was inoculated with the parent strain Aspergillus niger CA16 and the remaining one was inoculated with the mutant strain Aspergillus niger 79/20. All the flasks were then incubated for 15 days in an incubator under same conditions. Fermented media were collected on day 3, 6, 9, 12, 15 and were subjected to estimation of residual sugar, TTA value, citric acid concentration and pH determination.

III. Chemical Reagents and Solutions

All chemicals and reagents used in this study were of analytical grade. All aqueous solutions were prepared with distilled water. Stock solution of Prescott salt (NH4NO3: 2.23g/L, K2HPO4: 1.00g/L, MgSO4.7H2O: 0.23g/L) used in the media were prepared 10 times the media concentration and diluted as its normal strength in the experiment.

IV. Analytical Determination

At the different stages of fermentation the culture flasks were taken out of the incubator and the medium was collected onto the screw cap test tubes by pipetting and preserved at 0°C. The appropriate amount of sample was used for the estimation of total titratable acidity, citric acid and amount of residual sugar present in the medium after fermentation.

V. Determination of total Titratable Acidity (TTA)

Fermented medium (0.25ml) was diluted with 20ml of distilled water and was titrated against 0.1N NaOH solution using 2 to 3 drops of phenolphthalein as indicator. The value obtained was multiplied by 4 and total titratable acidity was expressed as ml of 0.1N NaOH required to neutralize 1ml fermented medium. The titrametric analysis of fermentation of each strain gave an indication of total acidity of the medium. The medium containing high TTA value i.e. higher acid content were then analyzed spectrophotometrically.

VI. Estimation of Citric Acid from Fermentation Medium

Citric acid was estimated spectrophotometrically by the reference method of Marier and Boulet (1958). Citric acid forms a color complex of polyvinyl keto-anhydridepolymer when it reacts with acetic anhydride and pyridine which can be estimated spectrophptometrically (Auterhoff and Schwingel, 1975). Following the growth of the organism aliquots of the medium were diluted so as to have concentration in the range of 25 to 200µg per ml (approximately) of citric acid.

VII. Estimation of Residual Sugar

Before inoculation and after completion of fermentation, samples were collected for initial and residual sugar estimation, respectively. Following the fermentation, amount of residual sugar in the medium was determined by diluting the aliquots of the medium so as containing sugar concentration range of 25-200 µg per ml.

Initial and residual sugar of the medium was determined spectrophotometrically by anthrone method (Morse, 1947) using anthrone as the coloring agent with sucrose as standard.

VIII. Results

a) Estimation of residual sugar at different periods of citric acid fermentation

The residual sugar concentration was different in various media during citric acid fermentation by
Aspergillus niger parent strain CA16 and mutant strain 79/20. Prescott salt was also found to have an effect on sugar concentration during citric acid fermentation. In the presence of Prescott salt, residual sugar found in the molasses fermentation medium by Aspergillus niger CA16 on day 3, 6, 9, 12 and 15 was 34.81, 26.38, 21.13, 17.75 and 14.64 mg/ml respectively and that in the absence of Prescott salt residual sugar found was 35.19, 30.25, 23.75, 15.23 and 10.92 mg/ml respectively. Lowest amount of sugar was found at Day 15 on molasses fermentation medium by Aspergillus niger CA16 in the absence of Prescott salt (Figure-15). Residual sugar found in the molasses fermentation medium by Aspergillus niger 79/20 on day 3, 6, 9, 12 and 15 was 36.64, 29.62, 21.98, 16.20 and 11.51 mg/ml respectively, in the presence of Prescott salt and 40.12, 34.41, 30.13, 25.55 and 17.51 mg/ml respectively in the absence of Prescott salt. At Day 15 lowest amount of residual sugar was found on molasses fermentation medium by Aspergillus niger 79/20 in the absence of Prescott salt which is higher than that found by Aspergillus niger CA16 in the absence of Prescott salt (Figure-14 & 15).

When Aspergillus niger CA16 used jackfruit (outer portion) as fermentation medium, the residual sugar found on day 3, 6, 9, 12 and 15 in presence of Prescott salt was 30.34, 24.34, 20.04, 16.78 and 12.32 mg/ml respectively and in the absence of Prescott salt was 32.55, 27.79, 20.13, 13.24 and 8.40 mg/ml respectively. Lowest amount of residual sugar was found in the absence of Prescott salt on the day 15 on the jackfruit (outer portion) fermentation medium by Aspergillus niger CA16 (Figure-15). In presence of Prescott salt, concentration of residual sugar on jackfruit (outer portion) fermentation medium by the mutant fungus Aspergillus niger 79/20 was 32.69, 28.43, 23.12, 19.24 and 14.34 mg/ml on day 3, 6, 9, 12 and 15 respectively. On the other hand, the residual sugar concentration was 36.73, 30.29, 26.51, 20.91 and 15.12 mg/ml during same incubation periods in the absence of Prescott salt. Lowest amount of residual sugar was found in the presence of Prescott salt on the day 15 in the jackfruit fermentation medium by Aspergillus niger 79/20 which is higher than that found by Aspergillus niger CA16 in the absence of Prescott salt (Figure-14 & 15).

On the mixed fermentation medium, residual sugar found in presence of Prescott salt was 36.14, 30.47, 22.81, 19.33 and 15.94 mg/ml respectively and in the absence of Prescott salt was 40.23, 33.021, 25.17, 16.35 and 12.13 mg/ml respectively on day 3, 6, 9, 12 and 15 by Aspergillus niger CA16. Lowest amount of residual sugar was found in the presence of Prescott salt on day 15 in mixed fermentation medium by Aspergillus niger CA16 (Figure-15). In presence of Prescott salt, residual sugar concentration was 38.81, 33.32, 29.62, 21.64 and 18.29 mg/ml respectively in mixed fermentation medium by Aspergillus niger 79/20 on day 3, 6, 9, 12 and 15. On the other hand, the residual sugar concentration in the absence of Prescott salt was 46.64, 41.69, 35.46, 29.60 and 20.12 mg/ml respectively on the same respective days. Lowest amount of residual sugar was found in the presence of Prescott salt on the day 15 in the jackfruit fermentation medium by Aspergillus niger 79/20 which is higher than that found by Aspergillus niger CA16 in the absence of Prescott salt (Figure-14 & 15).

Figure 14: Amount of Residual Sugar at different days of fermentation in various substrate (with Prescott salt) by A. niger CA16 and mutant strain A. niger 79/20.

Figure 15: Amount of residual sugar at different days of fermentation in various substrates (Without Prescott salt) by A. niger CA16 and mutant strain A. niger 79/20.

Figure 16: Amount of residual sugar at day 12 of fermentation in different substrates (with and without Prescott salt) by A. niger CA16 and mutant strain A. niger 79/20. Here, P & A indicates presence & absence of Prescott salt respectively.
On day 12, the residual sugar found on each of the three types fermentation media by the parent strain *Aspergillus niger* CA16 were somewhat lower in the absence of Prescott salt. On the other hand the residual sugar found on the same types of media by the mutant strain *Aspergillus niger* 79/20 were comparatively lower in the presence of Prescott salt on day 12 (Figure- 16).

b) Estimation of TTA values at different period of citric acid fermentation

Total titratable acidity (TTA) of different fermented media were determined after different incubation periods during citric acid fermentation by *Aspergillus niger* parent strain CA16 and mutant strain 79/20. In each case the TTA values were found to increase gradually with the increase in incubation periods from day 3 and picked on day 12 and then started to decrease (day 15) [(Figure -17, 18 & 19) and (Appendix-II & V)].

In the presence of Prescott salt, TTA value found in the jackfruit (outer portion) fermentation medium by *Aspergillus niger* CA16 on day 3, 6, 9, 12 and 15 was 0.2, 0.5, 1.2, 1.5 and 1.3 respectively and that in the absence of Prescott TTA value found was 0.3, 0.96, 1.5, 2.68 and 2.16 respectively. Highest TTA value was found at Day 12 on jackfruit fermentation medium by *Aspergillus niger* CA16 in the absence of Prescott salt. When *Aspergillus niger* 79/20 was the strain used for fermentation on jackfruit medium, the TTA value found in the presence of Prescott salt on day 3, 6, 9, 12 and 15 was 0.8, 1.2, 2.1, 2.9 and 2.5 respectively and in the absence of Prescott salt was 1.0, 2.1, 3.2, 4.6 and 4.22. TTA value was highest at day 12 in the absence of Prescott salt for *Aspergillus niger* 79/20 which was higher than that obtained for *Aspergillus niger* CA16 (Figure-17 & 18).

TTA value obtained on day 3, 6, 9, 12 and 15 was 0.3, 0.95, 1.6, 2.4 and 1.67 respectively in the presence of Prescott salt and in the absence of Prescott salt was 0.5, 1.2, 2.8, 3.4 and 2.85 respectively when the parent strain *Aspergillus niger* CA16 was allowed to ferment the mixed medium. Highest TTA value found in the absence of Prescott salt for *Aspergillus niger* 79/20 was 1.0, 1.89, 2.9, 3.4 and 2.95 respectively and in the absence of Prescott salt the TTA value was 1.2, 2.7, 3.95, 5.38 and 4.89 respectively. Once again the highest TTA value was obtained for *Aspergillus niger* 79/20 on day 12 in the absence of Prescott salt which was higher than that obtained in case of *Aspergillus niger*CA16 (Figure-17 & 18).
Optimization of Citric Acid Production by Substrate Selection using Gamma Ray Induced Mutant Strain of *Aspergillus niger*

Citric acid concentration obtained on day 3, 6, 9, 12 and 15 was 1.44, 3.13, 6.73, 9.62 and 7.26 mg/ml respectively in the presence of Prescott salt and in the absence of Prescott salt was 1.92, 3.85, 8.65, 14.33 and 11.29 mg/ml respectively when the parent strain *Aspergillus niger* CA16 was allowed to ferment the molasses medium. Highest citric acid concentration found at day 12 in the absence of Prescott salt for *Aspergillus niger* CA16 grown on molasses fermentation medium. When the same medium was fermented by *Aspergillus niger* 79/20, the citric acid concentration obtained in the presence of Prescott salt on day 3, 6, 9, 12 and 15 was 3.85, 6.25, 11.54, 14.90 and 12.98 mg/ml respectively and in the absence of Prescott salt the citric acid concentration was 5.29, 11.54, 16.83, 23.56 and 20.67 mg/ml respectively. Once again the highest citric acid concentration was obtained for *Aspergillus niger* 79/20 on day 12 in the absence of Prescott salt which was higher than that obtained in case of *Aspergillus niger* CA16 (Figure-20 & 21).

Accumulation of citric acid concentration on day 3, 6, 9, 12 and 15 was 0.96, 4.62, 7.21, 12.88 and 10.38 mg/ml respectively when the parent strain *Aspergillus niger* CA16 was allowed to ferment the molasses medium. Highest citric acid concentration found at day 12 in the absence of Prescott salt for *Aspergillus niger* CA16 grown on molasses fermentation medium. When the same medium was fermented by *Aspergillus niger* 79/20, the citric acid concentration obtained in the presence of Prescott salt on day 3, 6, 9, 12 and 15 was 3.85, 6.25, 11.54, 14.90 and 12.98 mg/ml respectively and in the absence of Prescott salt the citric acid concentration was 5.29, 11.54, 16.83, 23.56 and 20.67 mg/ml respectively. Once again the highest citric acid concentration was obtained for *Aspergillus niger* 79/20 on day 12 in the absence of Prescott salt which was higher than that obtained in case of *Aspergillus niger* CA16 (Figure-20 & 21).

**Optimization of Citric Acid Production by Substrate Selection using Gamma Ray Induced Mutant Strain of *Aspergillus niger***

These results showed TTA value was comparatively higher in the absence of Prescott salt for all the three types of media and for each of the strain. Throughout the incubation period the TTA value was highest in case mixed fermentation medium followed by molasses and jackfruit fermentation medium. Again, fermentation by *Aspergillus niger* 79/20 resulted in a comparatively higher TTA value than by *Aspergillus niger* CA16 both in the presence and absence of Prescott salt (Figure 19).

c) Estimation of citric acid accumulation at different period of citric acid fermentation

Accumulation of citric acid at different incubation periods on different media followed a very similar pattern as was seen in case of TTA value. Citric acid concentration was also different on different incubation periods with various fermentation media by the parent strain *Aspergillus niger* CA16 strain and the mutant strain 79/20. Citric acid concentration was found to increase gradually with the increase of incubation period and maximum citric acid concentration was found on day 12 in case of each of the three media. Finally, citric acid concentration was found to decrease at day 15 [(Figure-20, 21 & 22) and (Appendix-III & VI)].

On the jackfruit (outer portion) fermentation medium, citric acid concentration obtained in the presence of Prescott salt by *Aspergillus niger* CA16 on day 3, 6, 9, 12 and 15 was 0.96, 2.4, 5.77, 7.21 and 6.25 mg/ml respectively and in the absence of Prescott salt was 1.44, 4.62, 7.21, 12.88 and 10.38 mg/ml respectively. Highest citric acid concentration for *Aspergillus niger* CA16 was found in the absence of Prescott salt on 12 day. Citric acid concentration for mutant strain *Aspergillus niger* 79/20 on the same medium and on the same incubation periods was 3.85, 5.77, 10.09, 13.94 and 12.02 mg/ml respectively in the presence of Prescott salt and 4.29, 10.09, 15.38, 22.12 and 20.29 mg/ml respectively in the absence of Prescott salt. The highest citric acid concentration was obtained in the absence of Prescott salt for the mutant strain *Aspergillus niger* 79/20 which was higher than that found in case of parent strain *Aspergillus niger* CA16 (Figure-20 & 21).
In the presence of Prescott salt, citric acid concentration found in the mixed fermentation medium by *Aspergillus niger* CA16 on day 3, 6, 9, 12 and 15 was 1.44, 4.57, 7.69, 11.54 and 8.03 mg/ml respectively and that in the absence of Prescott citric acid concentration found was 2.40, 5.77, 13.46, 16.35 and 13.70 mg/ml respectively. Highest citric acid concentration was found at Day 12 on mixed fermentation medium by *Aspergillus niger* CA16 in the absence of Prescott salt. When *Aspergillus niger* 79/20 was the strain used for fermentation on mixed medium, the citric acid concentration found in the presence of Prescott salt on day 3, 6, 9, 12 and 15 was 4.29, 9.09, 13.94, 16.35 and 14.18 mg/ml respectively and in the absence of Prescott salt was 5.77, 12.98, 18.99, 25.87 and 23.51. Citric acid concentration was highest at day 12 in the absence of Prescott salt for *Aspergillus niger* 79/20 which was higher than that obtained for *Aspergillus niger* CA16 (Figure-20 & 21).

From the findings of this study it is clearly suggested that both fermentation medium and Prescott salt have a considerable effect on the production of citric acid. Among the media used in this study, the mixed fermentation medium was found to be most suitable for citric acid production followed by molasses and jackfruit (outer portion) media. Another important finding of the present study was that Prescott salt was found to have a negative effect on the citric acid production by the either strains of *Aspergillus niger*. Again the gamma-ray induced mutant strain, *Aspergillus niger* 79/20 had a yield efficiency more than that of the parent strain *Aspergillus niger* CA16 and thus considered superior to the parent strain *Aspergillus niger* CA16. Thus as far as citric acid production is concerned the mixed medium in the absence of Prescott salt is the most suitable medium and the gamma ray induced mutant strain *Aspergillus niger* 79/20 is the preferred organism.

**Figure 22:** Citric acid accumulation at day 12 of fermentation in different substrates (with and without Prescott salt) by A. niger CA16 and mutant strain A. niger 79/20. Here, P & A indicates presence & absence of Prescott salt respectively.

These results showed citric acid concentration was comparatively higher in the absence of Prescott salt for all the three types of media and for each of the strain. Throughout the incubation period the citric acid concentration was highest in case mixed fermentation medium followed by molasses and jackfruit fermentation medium. Again, fermentation by *Aspergillus niger* 79/20 resulted in a comparatively higher citric acid accumulation than by *Aspergillus niger* CA16 both in the presence and absence of Prescott salt (Figure-22).

Fermentation of citric acid for commercial production is dependent on many factors like quality of strains, nutritional composition of the media, environmental conditions, deficiency of manganese and other metals, pH, temperature and dissolved oxygen tension (Moyer, A. J. 1953). Usually, *Aspergillus niger* gives the best yield at around 25-28°C. Increase in incubation period resulted in the increased citric acid production. A lower concentration of sugar leads to lower yield of citric acid as well as accumulation of oxalic acid (Kovats, 1960). But the use of wild type strain of *Aspergillus niger* is not cost effective. So, high yielding strains were searched which will give the best yield at around the room temperature. The superior strains *Aspergillus niger* CA16 and gamma ray induced mutants *Aspergillus niger* 79/20 seem to have fulfilled the requirement. Thus these strains can be conveniently exploited for the production of citric acid from cane molasses, jackfruit (outer portion) and a mixture of the two substrates.

**References Références Referencias**


41. Maddox, I.S., Spencer, K.,Greenwood, J.M., Dawson, M,


**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.

© Copyright by Global Journals Inc.(US) | Guidelines Handbook
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 address 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 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. After certification of all your credentials by OARS, they will be published on your Fellow Profile link on website https://associationofresearch.org which will be helpful to upgrade the dignity.

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

The FARSM member also entitled to get the benefits of free research podcasting of their research documents through video clips. We can also streamline your conference videos and display your slides/ online slides and online research video clips at reasonable charges, on request.
The FARSM is eligible to earn from sales proceeds of his/her researches/reference/review Books or literature, while publishing with Global Journals. The FARSS can decide whether he/she would like to publish his/her research in a closed manner. In this case, whenever readers purchase that individual research paper for reading, maximum 60% of its profit earned as royalty by Global Journals, will be credited to his/her bank account. The entire entitled amount will be credited to his/her bank account exceeding limit of minimum fixed balance. There is no minimum time limit for collection. The FARSM member can decide its price and we can help in making the right decision.

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

MEMBER OF ASSOCIATION OF RESEARCH SOCIETY IN MEDICAL (MARSM)

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

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

MARSM accrediting is an honor. It authenticates your research activities. After becoming 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 benefits can be availed by you only for next three years from the date of certification.

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

As MARSM, you will be given a renowned, secure and free professional email address with 30 GB of space e.g. johnhall@globaljournals.org. This will include Webmail, Spam Assassin, Email Forwarders, Auto-Responders, Email Delivery Route tracing, etc.
We shall provide you intimation regarding launching of e-version of journal of your stream time to time. This may be utilized in your library for the enrichment of knowledge of your students as well as it can also be helpful for the concerned faculty members.

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

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

It is mandatory to read all terms and conditions carefully.
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 3 yrs.

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.
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.

© Copyright by Global Journals Inc.(US)| Guidelines Handbook
To avoid postal delays, all transactions are 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 hurdles 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 straightforward. 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.

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

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).

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

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

## A

Accumulation · 24, 25, 27
Amsterdam · 26
Aspergillus · 20

## C

Cytokeratins · 13, 14
Cytoplasm · 1, 3, 4

## E

Endocrine · 16, 18
Epithelium · 17
Ethanol · 27

## G

Glucoamylase · 20

## H

Hematoxylin · 14

## M

Masenyetsae · 12
Mediterranean · 10
Mesenchymal · 18

## O

Occurences · 10

## P

Pathogemc · 2
Perineural · 14, 15, 16
Phagocytic · 1
Pyridine · 21, 25

## R

Rifampicin · 7, 9

## S

Salicylic · 6
Spherical · 2

## T

Trophozoite · 1, 2