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Microbiology and Pathology

Acute Encephalitis Syndrome (AES)

Idiopathic Pulmonary Hemosiderosis

Highlights

Detection of Methicillin Resistant

Unilateral Primary Ovarian Lymphoma

Discovering Thoughts, Inventing Future

VOLUME 20 ISSUE 4 VERSION 1.0

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Acute Encephalitis Syndrome (AES): The Case Study of Muzaffarpur District of Bihar

By Mohammad Maaz Ahmad

Asfendiyarov Kazakh National Medical University

Abstract- Acute encephalitis syndrome (AES) is a serious public health problem in India specially Muzaffarpur district in Bihar. Bihar has recorded 188 cases of acute encephalitis syndrome, with 45 deaths since January. All casualties are children, the maximum in Muzaffarpur. Every year there is a repeat of the same, still no specific measures have been taken. The death of over 125 children in Muzaffarpur due to Acute Encephalitis Syndrome is very shocking.

These children come from low-income group families and are poorly nourished. It is time to remember Elizabeth Barrett Browning for her poem 'The Cry of the Children' dedicated to the condition of children in England who were made to clean chimneys and work in hazardous industries. As a result, many would catch serious diseases and eventually die an early death. The poem examines children's manual labor forced upon them by their exploiters. It was published in August 1843 in Blackwood's Magazine. However, since then England has moved far ahead. All the children go to school, get proper nutrition and health care required of them.

Keywords: Acute encephalitis syndrome (AES), chamki fever, Muzaffarpur district, child malnutrition, hypoglycaemic encephalopathy.

GJMR-C Classification: NLMC Code: WL 351

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Acute Encephalitis Syndrome (AES): The Case Study of Muzaffarpur District of Bihar

Mohammad Maaz Ahmad

Abstract- Acute encephalitis syndrome (AES) is a serious public health problem in India specially Muzaffarpur district in Bihar. Bihar has recorded 188 cases of acute encephalitis syndrome, with 45 deaths since January. All casualties are children, the maximum in Muzaffarpur. Every year there is a repeat of the same, still no specific measures have been taken. The death of over 125 children in Muzaffarpur due to Acute Encephalitis Syndrome is very shocking.

STATE B	RE	A	THES EASY
Comparative de till June 2019 (Ju	eaths ne 20	5)20)	
SKMCH	142	(6)	
KDKM	22	(1)	
Total	164	(7)	
Children admitt June 2019 (June	ted ti 202	ill D)	Color S
SKMCH	485	(49)	
KDKM	168	(8)	
Total	653	(57)	

These children come from low-income group families and are poorly nourished. It is time to remember Elizabeth Barrett Browning for her poem 'The Cry of the Children' dedicated to the condition of children in England who were made to clean chimneys and work in hazardous industries. As a result, many would catch serious diseases and eventually die an early death. The poem examines children's manual labor forced upon them by their exploiters. It was published in August 1843 in Blackwood's Magazine. However, since then England has moved far ahead. All the children go to school, get proper nutrition and health care required of them. However, where we stand today. The child malnutrition is one of the biggest problems in Bihar. Child malnutrition is an important public health problem in this northern state. According to the National Family Health Survey 2015to -2016, 48.3% of children are stunted, 20.8% are wasted, and 43.9% are underweight. In muzzafarpur district of Bihar, the most accepted cause is hypoglycaemic encephalopathy (known locally as Chamki fever). The children (aged 3-7 years) were from low-income families, had not eaten the previous evening, and had eaten unripe lychee in the morning. Lychee contains hypoglycin and methylenecyclopropylglycine (a toxic metabolite to mammals), which block the alternative pathway for glucose synthesis (fatty acid oxidation), causing a dramatic drop in blood glucose levels and metabolic hypoglycaemia.

Keywords: Acute encephalitis syndrome (AES), chamki fever, Muzaffarpur district, child malnutrition, hypoglycaemic encephalopathy.

INTRODUCTION

L

A clinical condition most widely caused by infection with Japanese encephalitis virus (JEV) or other infectious and noninfectious causes. Acute Encephalitis Syndrome (AES) Or Chamki fever is an inflammation of the brain outbreak of AES, which is called colloquially chmaki bukkhar (chamki fever), takes place in Muzaffarpur since 1996 almost every year. Malnutrition, climate, hygiene, inadequate health facilities, and lack of awareness are considered as contributing factors. This year, the main cause of death in most cases has been

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year, the main cause of death in most cases has been attributed to hypoglycaemia (low blood sugar level). Acute Encephalitis Syndrome (AES) is considered a very complex disease as it can be caused by various agents including bacteria, fungi, virus and many other agents. Acute Encephalitis Syndrome (AES) is considered a very complex disease as it can be caused by various agents including bacteria, fungi, virus and many other agents. It commonly affects children and young adults, resulting in considerable morbidity and mortality. Currently, the state of Bihar is seeing an outbreak of the deadly neurological disease, which has claimed lives of at least 156 children in Muzaffarpur and the adjoining districts. Cases of acute encephalitis syndrome have been observed mostly during April to June in Muzaffarpur, Bihar, particularly in children who are undernourished with a history of visiting litchi orchards, as per a report in *National Health Portal of India.* As a result of the outbreak, total 154 children died in the first three weeks of June 2019. A total of 440 cases of AES were admitted to hospital in these three weeks. At least 85 children of them died at the Sri Krishna Medical College and Hospital (SKMCH), the largest state-operated hospital in Bihar, while at least 18 children died at the Kejriwal Matrisadan, a trust-run hospital, in these weeks. Most of them were aged between 1 and 10 years.

In the subsequent months of July, August, and September; at SKMCH; 30, 18, and at least 12 cases were reported. Total 647 cases of AES including 161 deaths were reported between 1 June and 20 September 2019. The cause and manner of the disorder in a large number of AES cases still remain unidentified.



II. SIGN AND SYMTOMS

The signs and symptoms of AES include:

- 1. Clinical neurological manifestation that includes mental confusion, disorientation, delirium, or coma.
- 2. An acute onset of fever.
- 3. Headache.
- 4. Problems with speech or hearing
- 5. Stiff neck and back
- 6. Drowsiness
- 7. Hallucinations
- 8. Memory loss
- 9. Confusion
- 10. Vomiting

These are some symptoms of AES, when you see these symptoms immediately contact your doctor.

III. TREATMENT

The first treatment plan for this viral infection is hydration and increasing the glucose levels in the body. We can treat Acute Encephalitis Syndrome (AES) using antibiotics, antiviral medication, and supportive care. The treatment for hypoglycaemia includes supplying dextrose, a simple sugar similar to glucose, intravenously. "Nutrition and control of fever and seizures form the backbone of treating this disease. Some patients may also require breathing support and ICU care. In viral encephalitis, the antibiotics do not work and antivirals may be used." • The other treatment options are – bed rest, plenty of fluids, anti-inflammatory drugs to relieve the symptoms such as fever and headache. There is no cure for the disease. However, safe and effective vaccines are available to prevent encephalitis.

Vaccination: As per Govt. of India guidelines, 2 doses of JE vaccine have been approved to be included in UIP to be given one along with measles at the age of 9 months and the second with DPT booster at the age of 16-24 months w.e.f. April, 2013.

IV. Diagnosis

Laboratory-Confirmed case: A suspected case with any one of the following markers:

- Presence of IgM antibody in serum and/ or CSF to a specific virus including JE/Entero Virus or others
- Four fold difference in IgG antibody titre in paired sera
- Virus isolation from brain tissue
- Antigen detection by immunofluroscence
- Nucleic acid detection by PCR.

V. Prevention

- Personal preventive health measures such as the use of repellents, wearing long-sleeved clothes, practicing good hygiene - washing hands frequently and thoroughly with soap and water, especially after using the toilet, before and after meals - can help prevent viral encephalitis. Improve nutritional status of children at risk of JE/AES. Clothing reduces the risk of mosquito biting if the cloth is sufficiently thick or loosely fitting. Long sleeves and trousers with stockings may protect the arms and legs, the preferred sites for mosquito bites. School children should adhere to these practices whenever possible. Repellents are a common means of personal protection against mosquitoes and other biting insects. These are broadly classified into two categories, natural repellents and chemical repellents. Essential oils from plant extracts are the main natural repellent ingredients, i.e. citronella oil, lemongrass oil and neem oil.
- Govt. of India, as part of the National Programme for Prevention & Control of JE/AES, follows a multiencompassing pronaed strateav preventive (sanitation, safe drinking water, improvement in nutrition etc.), case management (capacity building of medical and para-medical staff, referral etc.) and rehabilitation (physical and social rehabilitation of disabled children), measures to address the problems relating to JE/AES. Doctors also suggest drinking plenty of water to stay hydrated, which supplies essential vitamins and flushes out any toxins from the body. The children return home in the evening and sometimes skip dinner. This leads

to night-time hypoglycaemia, does not sleep without eating dinner to prevent from this, *Malnutrition is the obvious result as poor families do not take healthy diet. Had they been taking a healthy diet, children would not have died in such large number.*

VI. CONCLUSION

Today one of the big problem face by Bihar is acute encephalitis syndrome (AES). The main problem of this disease is Poverty, malnutrition and lack of awareness. The logic behind the link between litchi fruit consumption and AES is that when children eat large amounts of unripe litchi fruits (on an empty stomach), it may lead to hypoglycemic encephalopathy. It is a that causes prolonged or severe hypoglycemia or low blood sugar. Unripe litchi has the toxins hypoglycin and methylene cyclopropyl glycine (MCPG) that cause vomiting if ingested in large quantities so, please do not eat litchi in an empty stomach. Well-nourished children are not affected by the consumption of Litchi fruit. AES affects only undernourished children who consumed litchi fruit the previous day and went to bed on an empty stomach.

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Molecular Detection of SV40, BKV and JCV in Esophageal and Colorectal Cancer Patients in Khartoum State, Sudan

By Musab D Hassan, Dalia Mursi Abdelhalim, Abdelrahim Mohamed Elhussein, Isam M Elkhidir & Khalid A Enan

Abstract- Background: The polyomaviruses that infect humans, BK virus (BKV), JC virus (JCV), and simian virus 40 (SV40), typically establish subclinical persistent infections. However, reactivation of these viruses in immunocompromised hosts is associated with renal nephropathy and hemorrhagic cystitis (HC) caused by BKV and with progressive multifocal leukoencephalopathy (PML) caused by JCV. Additionally, SV40 is associated with several types of human cancers including primary brain and bone cancers, mesotheliomas, and non-Hodgkin's lymphoma.

Aims: To study of SV40, BKV and JCV in Esophageal and Colorectal cancer Patients in Khartoum State, Sudan.

Objective: This study was designed to detect BKV, JCV, and SV40 among colorectal cancer and esophageal cancer patients in Khartoum State, Sudan during the period October 2018 to July 2019

Keywords: BKV, JCV, and SV40; colorectal cancer; esophageal cancer, khartoum state; Sudan.

GJMR-C Classification: NLMC Code: QW 160

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Methods: Paraffin embedded blocks of tumor specimen from 56 colorectal cancer patients and 25 from esophageal cancer patients were collected from Khartoum teaching hospital and Medical Military Hospital, Sudan. Multiplex nested PCR was used to investigate the presence of BKV, JCV, and SV40 viruses in these specimens.

Results: BKV, JCV, and SV40 DNAs were detected in 11 out of 56 (19.64%) colorectal cancer samples and 3 out of 25 (12%) esophageal cancer samples, . Out of 56 patients diagnosed with colorectal cancer patients, 17%, 1.75% were found positive for SV40, JCV respectively, but no BKV positive samples were detected. Out of 25 patients diagnosed with esophageal cancer patients, 8%, and 4% were found positive for JCV, and BKV respectively, but SV40 was not detected in any of these samples.

Conclusion: The incidence of BKV, JCV, and SV40 in colorectal and esophageal cancer patients in Khartoum State, was documented through the molecular detection of BKV, JCV, and SV40 indicating high prevalence rates for SV40 among colorectal cancer patients in Khartoum State. Detection of BKV, JCV, and SV40 using multiplex nested PCR was established. Generally, these findings are useful for future studies since there is little information available about BKV, JCV, and SV40 infection in Sudan.

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Keywords: BKV, JCV, and SV40; colorectal cancer; esophageal cancer, khartoum state; Sudan.

I. INTRODUCTION

Polyomaviruses are a family of non-enveloped DNA viruses with icosahedral capsids containing small, circular, double-stranded DNA genomes. Polyomaviruses have been isolated from multiple animal species including humans, monkeys, rodents, and birds. Each polyomavirus exhibits a very limited host range and does not usually productively infect other species (Fields et al., 1996, Imperiale, 2001).

The polyomavirus family includes several human viruses, JCvirus (JCV) and BK virus (BKV), both of which were isolated in 1971 from immune compromised patients (Shah, 1996). JCV was recovered from the brain of a patient with the initials J.C. who died of progressive multifocal leukoencephalopathy (PML), a demyelinating disorder of the central nervous system (CNS) (Padgett et al., 1971). BKV was isolated from the urine of a Sudanese renal transplant patient (with the initials B. K.) who developed ureteral stenosis and was shedding inclusion-bearing epithelial cells in his urine (Gardner et al., 1971).

In the late 1950s and early 1960s, millions of people around the world were inadvertently exposed to a third polyomavirus, Simian virus 40 (SV40) of rhesus macaques (*Macaca mulatto*), due to administration of contaminated polio vaccines (Mortimer et al., 1981). This virus, Simian virus 40 (SV40), is a natural infectious agent in rhesus macaque. Recent studies revealed the presence of SV40 DNA in healthy individuals that were never vaccinated with contaminated vaccines or those who had never been in contact with monkeys. Seroepidemiological studies revealed that up to 15% of the human population contains antibodies against Simian virus 40, thus supporting the possibility that SV40 can spread in human by means of horizontal infection and vertical transmission (Martini et al., 2007).

The most studied human polyomaviruses are BK virus and JC virus. The route of infection remains unknown, but respiratory, oral, body fluids, and renal tansplacental transmission has been suggested (Knowles, 2006). BKV is a nephrotropic virus, but nucleic

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acid sequences and proteins can be detected in other tissues like blood, brain, liver, heart, lung and gonads (Rekvig and Moens, 2002), while JCV nucleic acid can be found in the kidney, blood, urogenital system cells and the gastrointestinal tract (Dörries, 1984).

Adult seroprevalence for BKV and JCV is very high: more than 90% of the adult population is seropositive for BKV (Knowles et al., 2003), while 50 to 80% of adults have antibodies to JCV (Knowles, 2006, Khalili et al., 2007).

Interestingly, the antibody titer against BKV decreases as the age increases, while that of JCV remains relatively unchanged (Knowles, 2006, Dörries, 1984).

The primary infection with BKV and JCV seems to be asymptomatic and the virus establishes a harmless life-long latent infection in the host, but reactivation of the virus in immunosuppresed individuals can lead to illness. BKV is associated with nephropathy (PyVAN) in renal transplant patients and hemorrhagic cystitis (PyVHC) in bone narrow transplants (Fleischmann, 2009, Hirsch and Snydman, 2005).

JCV is causative agent of PML. a fatal progressive demyelinating disease of the central nervous system due to viral replication in the oligodendrocytes (Rekvig et al., 1997).

The polyomaviruses JCV, BKV, and SV40 have been implicated in several human diseases and are undergoing increased scrutiny as possible cofactors in human cancer (Ahsan and Shah, 2002). These viruses can induce tumors in several rodent species, and can be detected with higher frequency in certain tumors compared to the corresponding healthy tissue (Moens et al., 2011).

The first study of polyomavirus in Sudan was done in 2016, in symptomatic kidney transplant recipients (Helibi et al., 2016)⁻ The most recent study was done in 2017, in patients with Non-Hodgkin's Lymphoma (NHL) (Isam, 2017).

Antibody assays are commonly used to detect presence of antibodies against individual viruses. (Drachenberg et al., 2005), but is rarely used to detect primary infection since most primary infections occur asymptomatically in early childhood (Flaegstad. and Traavik, 1985, Bogdanovic et al., 1994), detection of polyomaviruses by PCR or multiplex nested PCR is more sensitive and useful, although it is possible to use electron microscopy and virus isolation (Padgett et al., 1971, Schmitt et al., 2011).

This present work aimed to provide a better understanding for the role of BKV, JCV and SV40 in colorectal and esophageal cancer patients in Sudanese populations and update the information regarding the disease situation due to lack of diagnostic tools in the Sudan.

II. MATERIALS AND METHODS

a) Study area

This study was conducted in Khartoum Hospitals (Royal care Hospital and Medical Military Hospital) during the period from, October 2018 to July, 2019.

b) Study design

This study is descriptive, cross- sectional study.

c) Ethical review

The study was approved by the Ethical Review Committee (ERC) of Al Neelain University, Faculty of Medical Laboratory Sciences. Informed consents were obtained from the patients.

d) Data collection method and tools

Through a structured questionnaire, information on age, gender, and type of tumor and place of sample collection was recorded.

e) Patient's inclusion criteria and sample size

Paraffin embedded blocks tumor specimens from 81 Sudanese patients (65 colorectal cancer and 25 Esophageal samples taken from normal and pathological lesion) were collected in sterile Eppendorf tube and stored at room temperature until used for DNA extraction. most frequent lesions were adenocarcinoma.

f) Sample deparaffinization

Tissue samples were deparaffinized using xylene dissolution, in brief two of 20 μ m sections were cut from each tissue sample block by the same person. To avoid cross-contamination, the microtome block was cleaned and the blade replaced between samples. All samples were deparaffinized by adding xylene for one hour and then washed by ethanol 100%, 80%, 60% and 40% conseeuctively then deionized water for 10 seconds for rehydration.

g) DNA extraction

DNA was extracted from rehydrated tissue by using DNA extraction Kit according to the protocol of the manufacturer (Analytikajena), Briefly, $20-\mu$ sections of rehydrated sample was added to 560 μ l buffer AVL, then incubated at room temperature for 10 minutes. Subsequently, 560 μ l of ethanol (96-100%) was added to the sample after which 630 μ l of the resulting solution was applied to a column. A volume of 500 μ l of AW1 and AW2 was added for washing and the nucleic acids were eluted with 60 μ l AVE buffer and stored at -20°C until used.

h) Multiplex nested Polymerase Chain Reaction (PCR)

The multiplex nested PCR was done as described by Bergallo (Bergallo et al., 2007), The test was carried out with first-round PCR amplification using the outer primer pairs that are specific for large T antigen gene to amplify a conserved DNA region of the large T antigen gene of JCV, BKV, and SV40.

The primers used consisted of forward primer 5'-TCYTCTGGNNTAAARTCATGCTCC-3' and reverse primer, 3'- CAAGGTATCCAACCKTRGATWAA -5'. The reaction was performed in 25µl volume using Maxime PCR PreMix Kit master mix (Intron. South Korea). The volume included: 5µl master mix, 1 µl forward primer, 1 µl reverse primer, 5 µl extracted DNA and 13µl distilled water. The DNA was amplified in thermo- cycling conditions using PCR machine (Techno ,Japan) as follow: initial denaturation at 94°C for 2 min, followed by 40 cycles of denaturation at 94°C for 1 min, annealing at 61°C for 1 min and extension at 72°C for 1 min, with a final extension 72°C for 5 min.

The second round was carried out using a set of primers designed to obtain products of different size for each related virus, inner primer pairs used consisted of BKV Sense 5'- GAATGCTTTCTTCTATAGTATGGTATG -3' and JCV Sense 5'- ATATTATGACCMCCAAAACCATG SV40 5'--3′ and Sense ATAATTTTTTGTATAGTAGTAGTAGTGCA -3' with reverse Polyomavirus Antisense 3'-CCTTTCAGRAAYCCCATAAGATGG-5' The reaction was performed in 25µl volume using Maxime PCR PreMix Kit master mix (Intron. South Korea).

The volume included: 5μ l master mix, 2μ l of primers mix included the inner primer pairs that mentioned above, 15μ l distilled water and 3μ l of first-round PCR product, second round was performed under the following conditions: 94° C for 30 s, 56° C for 1min, 72° C for 30 s for 30 cycles with a final extension 72° C for 5 min.

i) Visualization of products

10 μ l of the amplified product was subjected to direct analysis by gel electrophoresis in 2% Agarose, the gel was prepared by adding 1.6 g of Agarose to 75 ml 50X Tris Acetate EDTA buffer. The product was visualized by staining with 0.2 μ g/ml Ethidium bromide using UV gel documentation system Biometra (Germany). The expected size of SV40, BKV and JCV amplicons were 135 bp, 353 and 189 respectively.

j) Data analysis

Collected data were analyzed using the statistical package of social science (SPSS, version 12.0) program. Chi-square statistical analyses was used to determine P value significance range.

III. Results

a) Detection of JCV, BKV and SV40 in colorectal and esophageal cancer patients

Eleven out of 56 (19.64%) colorectal cancer samples were analyzed, 10 of which (17.85%) were found positive for SV40, and 1(1.78%) was found positive for JCV, BKV was not detected in any of the samples. (Table 3.1).

Three out of 25 (12%) esophageal cancer samples were analyzed, of which 1/25 (4%) was found positive for BKV and 2/25 (8%) were found positive for JCV, one of these sample showed mixed infections with BKV and JCV, however SV40 was not detected in any of the esophageal cancer patients. None of 25 normal lesion samples were positive for JCV, BKV and SV40. (Table 3.1).

b) The association between gender and the presence JCV, BKV and SV40 in colorectal and esophageal cancer patients

According to the gender for colorectal cancer patients JCV was detected in 1/34 (2.9%) male and SV40 was detected in 6/34 (17%) males and 4/22 (18.2%) of females.

In esophageal cancer patients BKV was detected in 1/16 (6.3%) female and JCV was detected in 2/16 (12.5%) females.

There was no significant association between the gender and virus detection neither for esophageal cancer (P value = 0.428) nor for colorectal cancer patients (P value = 0.135). (Table 3.2, Table 3.3)

c) The association between the age groups and presence of JCV, BKV and SV40 in colorectal and esophageal cancer patients

Based on age group, the distribution of JCV in colorectal cancer patients were 1/33 (3%) in the age groups 31-60 years old, while SV40 distribution of positive samples showed 2/7 (28.5%) in age groups 18-30 years old; 5/33 (15.5%) in age groups 31-60 years old; 3/16 (18.75%) in age groups 61-77 years old.

In esophageal cancer patients the age group distribution for JCV was 2/12 (16.6%) in age groups 31-60 years old, while distribution of BKV positive samples was 1/12 (8.3%) in age group 31-60 years old. No significant differences according to age were found (P value = 0.632) and (P value = 0.135) for colorectal cancer and esophageal cancer respectively. (Table 3.4), (Table 3.5).

Table 3.1: Detection of BKV, JCV and SV40 in colorectal and esophageal cancer

Polyomavirus	Colorectal	Esop	Total		
1 Olyomavirus	Olivieciai	Normal	pathological	TOLA	
BKV	0/56 (0%)	0/25 (0%)	01/25 (4%)	1/ 81(1.23%)	
JCV	01/56 (1.75%)	0/25 (0%)	02/25 (8%)	3/81 (3.7%)	
SV40	10/56 (17%)	0/25 (0%)	0/25 (0%)	14/81 (17.2%)	

Table 3.2: Patients with colorectal cancer, classified as to gender and the type of Polyomavirus in Khartoum State, Sudan 2018 (n=56)

Polyomaviruses	Male	Female	Total
BKV	0/34 (0%)	0/22 (0%)	0/56 (0%)
JCV	1/34 (2.9%)	0/22 (0%)	1/56 (1.78%)
SV40	6/34 (17%)	4/22 (18.2%)	10/56 (17.85%)
Total	34/56 (60.7%)	22/56 (39.3%)	11/56 (19.6%)

(P value = 0.428)

Table 3.3: Patients with esophageal cancer, classified as to gender and the type of Polyomavirus in Khartoum State, Sudan 2018 (n=25)

Polyomaviruses	Male	Female	Total
BKV	0 /9 (0%)	1/16 (6.3%)	1/25 (4%)
JCV	0/9 (0%)	2/16 (12.5%)	2/25 (8%)
SV40	0/9 (0%)	0 /16 (0%)	0/25 (0%)
Total	9/25 (36%)	16/25 (64%)	3/25 (12%)

(P value = 0.135)

Table 3.4: Patients grouped by age and the detected Polyomavirus in colorectal cancer patients in Khartoum State, Sudan 2018

Age groups	Col			
rige groupe	BKV	JCV	SV40	Total
18 – 30	0/7 (0%)	0/7 (0%)	2/7 (28.5%)	2/7 (28.5%)
31-60	0/33 (0%)	1/33 (3%)	5/33 (15.15%)	6/33 (18.1%)
61-77	0/16 (0%)	0/16 (0%)	3/16 (18.75%)	3/16 (18.75%)
Total	0/56 (0%)	1/56 (1.78%)	10/56 (17.85%)	11/56 (19.6%)

(P value = 0.632)

Table 3.5: Patients grouped by age and the detected Polyomavirus in esophageal cancer patients in Khartoum State, Sudan 2018

Age groups	Eso			
	BKV	JCV	SV40	Total
18 – 30	0/2 (0%)	0/2 (0%)	0/2 (0%)	0/2 (0%)
31-60	1/12 (8.3%)	2/12 (16.6%)	0/12 (0%)	3/12 (25%)
61-77	0/11 (0%)	0/11 (0%)	0/11 (0%)	0/11 (0%)
Total	1/25 (4%)	2/25 (8%)	0/25 (0%)	3/25 (12%)

(P value = 0.135)

IV. DISCUSSION

Oncogenic viruses may contribute to human carcinogenesis favoring genetic instability and inducing chromosomal aberrations (Duensing and Münger, 2003), it is well established that BKV, JCV, and SV40 can cause cancer in laboratory animals (Walboomers et al., 1999), and all three polyomaviruses are associated with human tumors. (Ahsan and Shah, 2006) however the role of polyomaviruses BKV, JCV and SV40 is still controversial. (White and Khalili, 2004).

The present study focused on the molecular diagnosis of three human polyomaviruses (BKV, JCV,

and SV40) in colorectal and esophageal cancer patients in Khartoum State, Sudan since little is known about the epidemiology of this three human polyomaviruses in Sudan in particular and in Africa in general.

The human polyomaviruses can persist in the host in a latent form and reactivate in the presence of immunosuppressive conditions. They are commonly associated with rejection of transplanted kidney (BKV) and progressive multifocal leukoencephalopathy (JCV). More recently, they have been linked to colorectal carcinogenesis. (Hori et al., 2005, Enam et al., 2002, Casini et al., 2005).

SV40 is a monkey virus that was probably introduced in the human population in the early 1960's by contaminated polio vaccines produced in monkey kidney cells where the virus can be present in a latent form. It probably continued to spread among humans through the sexual, haematogenic and orofecal routes, since it was found in urine and sewage samples. (Theodoropoulos et al., 2005, Li et al., 2002).

Colorectal cancer is one of the most common malignancies in developed countries. (Vastag, 2002), and this is the first report describing the presence of SV40 DNA in colorectal cancer in Sudan, we found that 17% of the sample were positive for SV40.These findings are similar of that reported by laura giuliani (2008) in Italy who reported that 15.1% of the colorectal cancer patients had the virus.

JCV was found positive in 1.75% of our clorectal cancer patients which is agreement with results 4.2% were positive for JCV DNA reported by El Hussein et al (2019) as well to that of Sarvari *et al* (2018) who reported low prevalence of 1.42% JCV DNA in Shiraz city, Iran, and lower percentage comparied to that reported by Enam et al (2002) who reported extremely high rate of 81% in colon cancers and to that of Rencic et al. (1996) who reported detection rate of 81.2% in colonic biopsy samples. The lack of detection of JCV T-Ag however cannot rule out a "hit and run" mechanism as demonstrated by Ricciardiello *et al* (2003). in an *in vitro* model of colonic cells BKV, on the other hand, was not detected in colorectal cancer.

For the esophageal cancer we found JCV DNA in 2/25 (8%) samples which slightly differs from that reported (53%) by Del Valle et al., (2005), and Ahsan and Shah, 2006).In our positive samples single infection was present in 1 case and dual infections in the remaining case which also had BKV, normal lesion samples were negative for JCV.BKV and SV40. The high prevalence of infection and detection of BKV and JCV in tonsils suggested that the virus is transmitted mainly by the respiratory route. However, it has been reported that JCV can infect cells in the tonsils and can spread from there by replication in lymphoid cells. (Ahsan and Shah, 2006).

BKV and JCV DNA sequences and virions are also detected in raw urban sewage, (Del Valle et al., 2005, Bofill-Mas et al., 2001) suggesting also a fecal-oral route of transmission for these viruses. In the present study, SV40 was not detected in any of the esophageal cancer specimens, other study in Sudan showed that prevalence of BKV and SV40 in NHL patient which both males are more susceptible (Isam et al., 2017).

The study showed that there is no significant association with gender or age with the presence of these three human polyomaviruses in both in colorectal and esophageal cancer.

Finally; our study showed the feasibility of Multiplex nested PCR assay for detection and

differentiation between JCV, BKV, and SV40 in cancer tissues and could be used for diagnostic purposes and epidemiological studies in the Sudan.

To our knowledge, this is the first attempt to detect JCV, BKV, and SV40 in colorectal and esophageal cancer patients in Sudan by using Multiplex nested PCR assay. The results obtained should call for wider surveillance at the national level in order to fully elucidate the true status and epidemiology of JCV, BKV, and SV40 in the country.

V. Conclusion

Incidence and existence of JCV, BKV, and SV40 in Sudan was documented through detection of these viruses DNAs in the tissue samples among colorectal cancer and esophageal cancer patients in Sudan, using Multiplex Nested PCR. Generally, these findings are useful for future studies since there is little available information about human polyomaviruses infection in Sudan.

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Unilateral Primary Ovarian Lymphoma: A Case Report

By Ayesha Afreen Islam

Abstract- Involvement of the ovary by malignant lymphoma is well known as a late manifestation of disseminated nodal disease. But primary ovarian lymphoma as the initial manifestation is unusual. Histologically they are almost always of the non-Hodgkin's type. Primary ovarian lymphomas, account for only 0.5% of all non-Hodgkin lymphomas (NHLs) and 1.5% of all ovarian neoplasms. Previous studies of NHL involving gynecologic sites have shown that most (90%) NHLs involving the ovary are systemic tumors, of which ovarian involvement is only one aspect. In this article we report a case of unilateral primary ovarian NHL in a 35 yrs female patient. Consecutive histopathology and immunohistochemistry study revealed a primary Diffuse Large B-Cell Lymphoma (DLBCL) of ovary.

GJMR-C Classification: NLMC Code: QZ 350



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Unilateral Primary Ovarian Lymphoma: A Case Report

Ayesha Afreen Islam

Abstract- Involvement of the ovary by malignant lymphoma is well known as a late manifestation of disseminated nodal disease. But primary ovarian lymphoma as the initial manifestation is unusual. Histologically they are almost always of the non-Hodgkin's type. Primary ovarian lymphomas, account for only 0.5% of all non-Hodgkin lymphomas (NHLs) and 1.5% of all ovarian neoplasms. Previous studies of NHL involving gynecologic sites have shown that most (90%) NHLs involving the ovary are systemic tumors, of which ovarian involvement is only one aspect. In this article we report a case of unilateral primary ovarian NHL in a 35 yrs female patient. Consecutive histopathology and immunohistochemistry study revealed a primary Diffuse Large B-Cell Lymphoma (DLBCL) of ovary.

I. INTRODUCTION

on-Hodgkin's lymphoma (NHL)is known to involve the female genital tract. The ovary is the most common anatomic site to be involved. Ovarian involvement by NHL is usually secondary, occurring as a part of systemic disease. ^[1] The primary involvement of the ovary by NHL is rare and accounts for less than 0.5% of all ovarian neoplasms, unlike testis where primary lymphomas account for 5% of testicular neoplasms. Less than 10% of all ovarian NHLs have been reported to be of primary origin. ^[2] Their presentation is similar to other ovarian tumors and less commonly, the tumors are incidental findings.^[3]

Here, we take the opportunity to report a case of ovarian NHL along with the immuno histochemical (IHC) panel and its differential diagnosis.

II. CASE REPORT

A 45 yr old female presented to the gynecology OPD with complaints of amenorrhea, occasional spotting, distension of abdomen and pain in the lower abdomen. Her sonogram revealed a right adnexal mass measuring 51mmx37mmx10mm (Fig1A). The SOL was solid cystic in nature. The left ovary was unremarkable. Pelvic lymph nodes were not involved (Fig 1B). The patient underwent total abdominal hysterectomy with bilateral salphingo-oopherectomy. On histopathological examination, the uterus and the smaller left ovary were unremarkable. Sections from the right ovary showed a tumor with solid and cystic areas comprising sheets of small round cells with hyper chromatic nuclei, 2-3 nucleoli and scanty basophilic cytoplasm. The tumor was interspersed with plentiful tingible body macrophages. The capsule was intact. (Fig2A,2B,2C). Although the tumor morphologically resembled a lymphoma, in view of the common differentials like the possibility of a poorly differentiated carcinoma had to be excluded. A panel of IHC for markers was used. The tumor showed to be LCA positive, CD20 positive (Fig 2D) and was negative for CK, inhibin and CD-117. PAX8 and WT1 were also negative, ruling out the possibility of ovarian carcinoma, dysgerminoma and desmoplastic small round cell tumor. Ki 67 index was approximately 10%.

Based on histology and IHC results the diagnosis of unilateral primary DLBCL of the ovary was made.

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III. Discussion

Primary ovarian NHL often mimics the more common ovarian tumors like advanced epithelial carcinoma therefore correct diagnosis is needed for ideal treatment. ^[3] Secondary ovarian involvement by malignant lymphoma is a well recognized entity and has been reported in 20-30% of cases in some autopsy series. The distinction between primary and secondary lymphomas is usually made postoperatively, after thorough histological examination and ruling out secondary involvement. In this study the confirmation of the diagnosis was made postoperatively, as the preliminary diagnosis was an ovarian carcinoma. As suggested by Fox et al, diagnostic criteria for primary ovarian lymphoma are a) a disease confined to ovary, b) absence of disease in blood and bone marrow; c) the extra ovarian carcinoma deposits if any should appear at least after few months.^[4] In our case there was no blood, bone marrow, spleen or hepatic involvement. Other sided ovary and fallopian tube were unremarkable. Absence of lymphadenopathy with normal blood and bone marrow findings favor the diagnosis of primary lymphoma.^[4] Although CA-125 is a sensitive marker, it lacks specificity for confirming the diagnosis of epithelial ovarian tumors. High serum levels of CA-125 have been reported sometimes in ovarian lymphoma.^[1]

Histologically, nearly all primary lymphomas (80%-95%) of the ovary are B-cell neoplasms. DLBCL is most common in the 35 to 45year age group, and our case falls into this age group. Rarely patients of ovarian lymphoma are HIV positive and immuno suppressed similar to cases of primary testicular and primary CNS lymphomas, however in majority of the cases there are no predisposing factors.^[5] Primary lymphoma of the ovary may be a misdiagnosed with other primary ovarian tumors like dysgerminoma, granulocytic sarcoma, granulosa cell tumor, undifferentiated carcinoma, Desmoplastic Small Round Cell Tumor (DSRCT) and metastatic carcinoma. Of these, dysgerminoma and DSRCT commonly mimic lymphoma, both macroscopically and microscopically. Only 10% of dysgerminoma are bilateral in contrast to 50% of the lymphomas.^[4,6] The most common histological subtype of Primary Ovarian NHL in adults is DLBCL (75%) followed by Burkitt lymphoma (12.5%) and Follicular Lymphoma and rare cases of B and T lymphoblastic lymphoma. Burkitt lymphoma is the most common NHL subtype affecting ovaries of children and adolescents.^[8] Histomorphological features of the tumor cells along with IHC help to arrive at a definite diagnosis. Immunohistochemistry in our case showed positivity of tumor cells for B lineage markers (CD 20) and negative for epithelial and mullerian markers.^[9]

Primary lymphomas distinctly have a better prognosis than poorly differentiated ovarian carcinomas. Therefore, it is important to make the distinction of this entity from other differentials. Treatment principles and prognosis are same as that of other nodal lymphomas and there are documentation of complete remission after treatment with chemotherapeutic regimens.^[1] However, after documentation of complete remission, the patient should be assessed clinically (history and physical examination) at 3-month follow-ups for 2 years, every 6 months for the next 2 years, and yearly thereafter. Repeat contrast-enhanced CT or PET-CT should be per-formed at follow-up only if there is a clinical suspicion of relapse. ^[1] The follow-up of the patients with primary ovarian lymphoma remain the same as for nodal lymphomas.^[10]

IV. Conclusion

Here we take the opportunity to report a case of Primary NHL of the ovary along with its histological differentials. IHC is useful in establishing and categorizing the tumor subtype. Accurate diagnosis and subtyping is important for management as its prognosis differs vastly from other primary and metastatic ovarian tumors.

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Epidemiology of *Staphylococcus spp.* with Analysis of Various Available Methods for Detection of Methicillin Resistant Staphylococcus Aureus

By Gitali Bhagawati, Sania Paul, Rekha Saji Kumar, Mansi & Khushboo Abstract- Staphylococcus aureus is one of the major resistant pathogens in clinical practice; Methicillin Resistant Staphylococcus aureus (MRSA) has come out as superbugs. Apart from this, with the increase in the number of hospitalized immunocompromised patients, Coagulase negative Staphylococcus (CONS) have become a major cause of nosocomial infections. Although molecular method like mecA gene detection is gold standard for MRSA, minimum inhibitory concentration (MIC) of cefoxitin or oxacillin can also be considered as standard where molecular methods are not available. Cefoxitin 30 µg disc or PBP 2a agglutination test can also be used as standard marker for MRSA identification. In this study, out of total 184 clinically significant, non-duplicate specimens, 150 (81.52%) isolates were Staphylococcus aureus and 34 (18.48%) were CONS. Among the CONS, the predominating isolate was Staphylococcus haemolyticus 15 (44.12%), followed by Staphylococcus epidermidis 10 (29.41%). In our study, cefoxitin disk diffusion test was found to have sensitivity 100%, specificity 92.15% and negative predictive value (NPV) 100%. PBP2a latex agglutination test was found to have sensitivity 99%, specificity 97.87% and negative predictive value (NPV) 97.87% in our study. In both the methods, MIC of cefoxitin has been considered as gold standard.

GJMR-C Classification: NLMC Code: WQ 4

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Epidemiology of *Staphylococcus spp.* with Analysis of Various Available Methods for Detection of Methicillin Resistant Staphylococcus Aureus

Gitali Bhagawati ^a, Sania Paul ^o, Rekha Saji Kumar ^o, Mansi ^w & Khushboo [¥]

Abstract- Staphylococcus aureus is one of the major resistant pathogens in clinical practice; Methicillin Resistant Staphylococcus aureus (MRSA) has come out as superbugs. Apart from this, with the increase in the number of hospitalized immunocompromised patients, Coaqulase negative Staphylococcus (CONS) have become a major cause of nosocomial infections. Although molecular method like mecA gene detection is gold standard for MRSA, minimum inhibitory concentration (MIC) of cefoxitin or oxacillin can also be considered as standard where molecular methods are not available. Cefoxitin 30 µg disc or PBP 2a agglutination test can also be used as standard marker for MRSA identification. In this study, out of total 184 clinically significant, non-duplicate specimens, 150 (81.52%) isolates were Staphylococcus aureus and 34 (18.48%) were CONS. Among the CONS, the predominating isolate was Staphylococcus haemolyticus 15 (44.12%), followed by Staphylococcus epidermidis 10 (29.41%). In our study, cefoxitin disk diffusion test was found to have sensitivity 100%, specificity 92.15% and negative predictive value (NPV) 100%. PBP2a latex agglutination test was found to have sensitivity 99%, specificity 97.87% and negative predictive value (NPV) 97.87% in our study. In both the methods, MIC of cefoxitin has been considered as gold standard.

I. INTRODUCTION

taphylococcus aureus is one of the major resistant pathogens in clinical practice. Methicillin Resistant Staphylococcus aureus (MRSA) is defined as a strain of S. aureus that is resistant to a large group of antibiotics called β -lactams, that includes penicillins and cephalosporins.¹ The first case of MRSA was reported in Britain in 1961 and is now "quite common" in hospitals.¹, ²Methicillin resistance in *S. aureus* is primarily mediated by overproduction of PBP2a protein, an altered penicillin-binding protein with lower affinity for betalactam antibiotics than PBP2, the main physiological methicillin target. PBP2a is encoded by the mecA gene, a component of a larger DNA fragment designated the mec region.^{1, 3, 4} Coagulase negative Staphylococcus (CONS) have been considered as non-pathogenic and were rarely reported to cause severe infections. However, with the increase in the number of hospitalized immunocompromised patients, CONS have become a major cause of nosocomial infection and they account for 9% of these infections.⁵

There are many traditional and commercial systems for detection of MRSA in clinical microbiology laboratories. Until 2006, Oxacillin disc and agar screening methods were used for detection of MRSA, however, in January 2006, Clinical Laboratory Standards Institute (CLSI) recommended use of Cefoxitin 30 µg disc as standard marker for MRSA identification.⁶ The shift towards use of Cefoxitin disc is emphasized because of its property to induce production of PBP2a in-vitro, thus it has better predictive value for detection of hetero-resistance in MRSA isolates.⁷ The gold standard method for antimicrobial susceptibility testing has been the minimum inhibitory concentration (MIC) test determined by dilution methods. In the recent years, MIC methods have been replaced by molecular methods which detect mecA gene as a gold standard for determining classical methicilin resistance in S. aureus. However, the use of molecular methods for detection of MRSA is largely restricted to reference laboratories and is not utilized in many microbiology laboratories as a routine test.²

The aims and objectives of the study were:

- 1. To detect the prevalence of *Staphylococcus aureus* and clinically significant CONS in various clinical specimens
- 2. Speciation of CONS
- To isolate MRSA by easily available phenotypic methods: MIC level detection of Cefoxitin/Oxacillin, Disk diffution of Cefoxitin 30 µg disc and PBP 2a agglutination test.
- To evaluate the sensitivity, specificity, positive predictive value (PPV) and negative predictive value (NPV) of these methods for identification of these strains.

Since PCR was not available for routine tests in the laboratory, MIC level detection of Cefoxitin/Oxacillin was considered as a gold standard.

II. MATERIALS AND METHOD

This prospective study was done in the Department of Microbiology in a tertiary care hospital in Delhi over a period of one year. Isolates of *Staphylococcus aureus* and CONS were collected from various clinical samples that were grown in routine cultures. The clinical specimens comprised of pus, blood, urine, high vaginal swab (HVS), body fluid,

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endotracheal (ET) tube secretion, discharge from eye and ear, joint aspirate and Central venous catheter line (CVP) tip. A total of 184 consecutive, non-duplicate, clinically significant isolates were collected for this study.

a) Bacterial identification and antimicrobial susceptibility testing

The clinical specimens were inoculated on 5% sheep blood agar and MacConkey's agar (HiMedia, New Delhi, India), incubated at 37°C for 24-48 h, and examined for bacterial growth. The identification was done by manual as well as by Automated System (Vitek 2 Compact System, bioMérieux). Manual methods were based on colony morphology, Gram's stain, catalase test, mannitol fermentation, and coagulase test (slide and tube method). All the isolates were subjected to three methods of identification of methicillin resistance:

- MIC breakpoints of oxacillin given by Vitek 2 Compact system or MIC level detection of Cefoxitin by E-test (Himedia). *Staphylococcus aureus* ATCC 29213 were used as control for MIC level detection.
- 2. Modified Kirby-Bauer disk diffusion method using Cefoxitin disks (30µg) on Mueller-Hinton agar (MHA). MHA plates were overlaid with clinical strain of the S. aureus with an inoculum of 0.5 McFarland turbidity standards. Cefoxitin 30 µg discs were used and incubated at 35°C for 24 hours. Cut off zone diameters for Cefoxitin was according to CLSI 2015. For quality control, ATCC controls strains for MRSA and MSSA were placed on the same plate.

- 3. PBP2' Latex agglutination test. (Oxoid, ThermoFisher Scientific, Basingstoke, England).
- b) PBP2' Latex Agglutination test

A loop-full of organisms was placed into a microcentrifuge tube with 4 drops of Extraction Reagent 1; the tubes were then placed in a heating block (>90°C), and after 5 minutes, the tubes were removed and allowed to cool to room temperature. A single drop of Extraction Reagent 2 was added to each tube, mixed well, and centrifuged at 1,500g for 5 minutes. The supernatant, 50 μ L, was used for testing with 1 drop of the latex particles. The supernatant and latex particles were mixed together with a stick, and the test card was rocked for 3 minutes. Tests were read visually. Agglutination of the test but not the control latex was considered positive, while no agglutination was considered negative.

The data obtained was recorded on Microsoft excel (2007 version) and analyzed. The results are explained in frequency (number) and in percentage (%).

III. Results

Out of total 184 clinically significant, nonduplicate specimens, 150 (81.52%) isolates were *Staphylococcus aureus* and 34 (18.48%), were CONS. Among the CONS, the predominating isolate was *Staphylococcus haemolyticus*15 (44.12%), followed by *Staphylococcus epidermidis*10 (29.41%) (*Fig1*)



Out of total 184 Gram positive cocci, 104 (56.52%) were isolated from males and 80 (43.48%) from female patients. *(Table1)*

Staphylococcus aureus strains were isolated from106 (70.66%) indoor patients, followed by 29 (19.33%) intensive care unit (ICU) and neonatal ICU (NICU) patients. Among the clinically significant CONS, 23(67.65%) were isolated from indoor patients and the rest 11(32.36%) were from ICU and NICU patients. (*Table1*)

Isolates	Male	Female	ICU	NICU	Indoor	Outdoor
Staphylococcus aureus(150)	84	66	16	13	106	15
S. haemolyticus(15)	8	7	2	4	9	0
S. epidermidis(10)	6	4	2	0	8	0
S. hominis. hominis(4)	3	1	0	2	2	0
S. xylosus(3)	3	0	0	0	3	0
S. arlette(1)	0	1	0	1	0	0
S. simulans(1)	0	1	0	0	1	0
TOTAL(184)	104	80	20	20	129	15

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Overall, the predominating specimen of isolating the Gram positive cocci was found to be pus 105 (57%), followed by blood 57(31%). Specimen wise distributions of *Staphylococcus aureus* and CONS have been shown in *Table2*.

Specimen Isolates	Pus	Blood	Urine	High Vaginal Swab	Body Fluid	ET secretion	Eye Discharge	Ear Discharge	Joint Aspirate	CVP tip
Staphylococcus aureus (150)	86	45	9	4	1	2	2	0	1	0
S. haemolyticus (15)	9	4	0	0	0	0	0	0	1	1
S. epidermidis (10)	5	4	0	0	0	0	0	1	0	0
S. hominis. hominis (4)	2	2	0	0	0	0	0	0	0	0
S. xylosus (3)	2	1	0	0	0	0	0	0	0	0
S. arlette (1)	0	1	0	0	0	0	0	0	0	0
S. simulans (1)	1	0	0	0	0	0	0	0	0	0
Total	105	57	9	4	1	2	2	1	2	1

Table 2: Specimen wise distribution of isolate

All the isolates of *Staphylococcus aureus* were subjected to three phenotypic methods of identifying methicillin resistance. Considering MIC level as gold standard, Cefoxitin disk diffusion test was found to have sensitivity 100%, specificity 92.12% and negative predictive value (NPV) 100% while PBP2a latex agglutination test was found to have sensitivity 99%, specificity 97.87% and negative predictive value (NPV) 97.87%.

Table 3: Comparison between different phenotypic methods considering MIC level as Gold Standard in relation to MRSA

Phenotypic Methods	Result	MIC Level: Resistant	MIC Level: Susceptible	Sensitivity	Specificity	Positive Predictive Value (PPV)	Negative Predictive Value (NPV)
PBP2'a Latex	Positive	102	1	00%	07.079/	99%	97.87%
Agglutination Test	Indeterminate/ Negative	1	46	99%	97.87%		
Cefoxitin 30	Resistant	99	0	1000/			100%
μ g Disk Diffusion	Susceptible	4	47	100%	92.15%	96.12%	

IV. DISCUSSION

Among the Gram-positive pathogens, S. aureus continues to cause skin and soft tissue infections (SSTI) in the community as well as invasive infections in the hospitalized patients.9 In our study, out of total 184 clinically significant, non-duplicate (except blood) specimens, 150 (81.52%) isolates were S.aureus. The most common clinical sample from which S. aureus have been isolated was pus or wound swabs 86 (57.33%). [Fig 1, Table 2] One similar finding corresponded S.aureus 165, out of which out of 131 (79.39%) were from pus samples.¹ In a Europian survey, the most common organisms in skin and soft tissue infections (SSTI) were S. aureus (71% cases) with 22% being MRSA.^{10, 11}

In a study from Germany, out of 1037 bacteraemic episodes in children over 10 years, Grampositive bacteria accounted for two third of all episodes in paediatric patients.¹² In another study from UK, out of 131 episodes of blood stream infection in a paediatric ICU over a period of 3 years, 63% was because of Gram-positive organisms.¹³ In our set up, bacteraemia due to Gram positive cocci have been isolated in 57(31%) cases over a period of one year which is corresponding with above mentioned studies. However, our finding is in contrast to one Indian study [7 (4.24%)].¹

Coagulase Negative Staphylococci (CONS) form a part of the normal commensal flora. To know the pathogenic potential, speciation of CONS is necessary. Out of total 184 clinically significant samples 34 (18.48%) were CONS. Among the CONS, the predominating isolate was Staphylococcus haemolyticus 15 (44.12%), followed by Staphylococcus epidermidis 10 (29.41%) [Fig1, Table 2]. This corresponds to other findings for S. epidermidis, 30.72%¹⁴ and 44.8%⁵. Isolation rate of Staphylococcus haemolyticus 23.84%¹⁴ and 19.7%⁵ are not corresponding to our findings. Out of total 15 isolates of Staphylococcus haemolyticus, 9 (60%) isolates were from pus or wound swab, followed by blood 4(11.76%). [Table 2] This is almost similar to another study, Staphylococcus haemolyticus, 6 (13%) in blood and 7 (7.3%) in skin infection.¹⁵

Our study shows isolation rate of MRSA by cefoxitin disc diffusion was 99 (66%).[Table 3] This is similar to the study done by R. Kaur⁴ in which out of 97 S. aureus strains, 53 (56.64%) were MRSA. The study done by INSAR¹⁶ also shows similar pattern of resistance, 42 % in 2008 and 40% in 2009. The prevalence of MRSA varies between regions and between hospitals in the same region as seen in a study from Delhi, where the MRSA prevalence in nosocomial SSTI varied from 7.5 to 41.3 % between three tertiary care teaching hospitals.¹⁶ The cause of varied prevalence rate of MRSA depends on multiple factors like proper sample collection, monitoring of infection control protocol implementations like hand hygiene protocol, barrier nursing or isolation policy, antibiotic policy of the hospital, prophylaxis policy protocol etc.

In our study, isolation rate of MRSA as per PBP2 a latex agglutination test was 102 (68%) [Table 3]; this is similar to findings of other studies, 42.4%¹⁷ and 45.36%⁴.

In our study, cefoxitin disk diffusion test was found to have sensitivity 100%, specificity 92.15% and negative predictive value (NPV) 100%. [Table 3] This is similar to study (sensitivity 100%, specificity 96.23% and NPV 100%)⁴ but dissimilar to other studies (sensitivity 92% and specificity 98%)³ and (sensitivity 90.9% and specificity 98.2%).¹⁷

Authors revealed in their study that low level Oxacillin resistance was detected better by Cefoxitin DD test.18

PBP2a latex agglutination test was found to have sensitivity 99%, specificity 97.87% and negative predictive value (NPV) 97.87% in our study. [Table 3] This is in concordance with 97.6% sensitivity with this assay.¹⁹ In one study, the authors have mentioned PBP2a latex agglutination 100% correlation with the oxacillin MIC which is almost similar with our finding.²⁰ Our finding is in contrast to another finding, sensitivity 100%, specificity 100% and NPV 100%⁴.

LIMITATIONS OF THE STUDY

The limitation of the present study is that it mec A gene could not be detected among the isolates.

V. CONCLUSION

To know the prevalence of Gram positive cocci, Staphylococcus aureus along with MRSA in a hospital set up is an urgent need so that the spread of resistant strains can be controlled in that environment. Speciation of CONS, mainly in immunocompromised patients helps us to learn about diversity, epidemiological pattern and virulence. Correlation with patient's clinical status adds to the diagnosis. Proper quality control of the microbiological testing methods including Gram's staining to check the arrangements of Gram positive cocci, agglutination in coagulase testing, 0.5 Mac Farland Standard during Antimicrobial Susceptibility testing and measuring zone sizes according to CLSI guideline taking ATCC strains as control should not be subjective. Standardisation in each step can detect the resistant strains by these fast and effective methods which are easily available and applicable without having the facility of detection of mecA gene.

Conflict of interest: None

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Gastric Lavage-An Alternate Approach in the Diagnosis of Idiopathic Pulmonary Hemosiderosis: A Case Report

By Ayesha Afreen Islam

Abstract- Idiopathic pulmonary Hemosiderosis (IPH) is a rare and life threatening condition, found primarily in children, that causes recurrent episodes of diffuse alveolar hemorrhage. It is characterized by hemoptysis, alveolar infiltrates on chest radiograph and various degrees of anemia. It affects pediatric patients in approximately 80% of cases with equal gender incidence. When no underlying cause for repeated episodes of diffuse alveolar hemorrhage is apparent, the entity is referred to as idiopathic pulmonary Hemosiderosis. Studies have shown that the detection of hemosiderin-laden macrophages in broncho alveolar lavage fluid and gastric aspirate is crucial for the confirmation of diagnosis. We present a case of 10 months old baby boy where cytological findings in the gastric lavage proved highly beneficial in the diagnosis of IPH.

GJMR-C Classification: NLMC Code: WG 142



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Ayesha Afreen Islam

Abstract- Idiopathic pulmonary Hemosiderosis (IPH) is a rare and life threatening condition, found primarily in children, that causes recurrent episodes of diffuse alveolar hemorrhage. It is characterized by hemoptysis, alveolar infiltrates on chest radiograph and various degrees of anemia. It affects pediatric patients in approximately 80% of cases with equal gender incidence. When no underlying cause for repeated episodes of diffuse alveolar hemorrhage is apparent, the entity is referred to as idiopathic pulmonary Hemosiderosis. Studies have shown that the detection of hemosiderin-laden macrophages in broncho alveolar lavage fluid and gastric aspirate is crucial for the confirmation of diagnosis. We present a case of 10 months old baby boy where cytological findings in the gastric lavage proved highly beneficial in the diagnosis of IPH.

I. INTRODUCTION

he estimated incidence of IPH in children is 0.24-1.23 cases per million, with a mortality rate as high as 50%. Only 500 cases have been described in medical literature.^[1] It is characterized by the triad of iron deficiency anemia, hemoptysis, and multiple alveolar infiltrates on chest radiograph.^[2,3] It may occur as a primary phenomenon, most commonly seen in children or secondary to cardiac, systemic vascular or hemorrhagic diseases, which is more common in adults.^[3] IPH is a diagnosis of exclusion and there should be a high degree of suspicion if patients with iron deficiency anemia don't respond to iron therapy, they should be examined for IPH.^[4] Laboratory and radiological findings that have been found helpful in diagnosing the disease are anemia(the value of RBC indices, and serum iron were very low, peripheral smear examination shows microcytic hypo chromic anemia, bone marrow biopsy show hyper plastic erythropoiesis), chest X-ray showing diffuse parenchymal infiltrates, pulmonary function tests showing interstitial lung disease, and increase in single breath carbon monoxide (CO) uptake.^[5] A diagnosis by lung biopsy is considered gold standard but many have accepted the presence of hemosiderin laden macrophages in gastric or broncho alveolar lavage fluid as diagnostic if other diagnostic criteria are met.^[1] The definitive diagnosis of IPH is made based upon the typical clinical and radiological profile, accompanied by the identification of hemosiderin-laden macrophages in sputum or in gastric lavage.^[1,4]

II. CASE REPORT

A 10 month old baby boy presented to the pediatric OPD with complains of failure to thrive, blood stained sputum and recurrent episodes of hemoptysis and severe pallor which was refractory to extensive iron therapy he received in the past. On clinical examination the patient was afebrile, no organomegaly and patients' hemogram showed severe microcytic hypo chromic anemia. On admission patient had pallor +++, bilateral crepitations on chest examination, other systems were unremarkable. Chest X-ray showed bilateral infiltrates (Fig 1). Anti GBM antibody was negative. A spectrum of other antibodies like ANCA, ANA, APLA, MPO and PR-3 all proved to be negative in this case. C-reactive protein, rheumatoid factor, antinuclear antibody (ELISA), anti phospholipid antibody were all negative. AFB and CBNAAT study in the sputum and gastric lavage were also negative. A diagnosis of Idiopathic pulmonary hemosiderosis was made based on the clinical triad (iron deficiency anemia, hemoptysis, bilateral chest infiltrates) and after ruling out other causes of alveolar hemorrhage by relevant investigations. A Cytological study of the gastric lavage revealed numerous hemosiderin-laden macrophages (HLM) confirmed by Perl's Prussian blue stain for hemosiderin (Fig 2A, 2B). Broncho alveolar lavage was not possible in our case owing to young age of the patient. The case was diagnosed as Idiopathic pulmonary hemosiderosis and patient was treated with systemic steroids. The patient is clinically stable at present.

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Fig. 1: X ray showing bilateral infiltrates.



Fig. 2(A, B): Perl's Prussian blue stain showing hemosiderin pigment.

III. DISCUSSION

Studies have shown that the age of presentation in IPH is bimodal, with a frequency peaks in children less than five years of age and in adolescents 11 years or older.^[2] Our patient was a 10 month old baby boy. Bulucea C et al in their study of IPH in the Romanian population found that 4 out of the 15 children diagnosed presented with the classical triad of clinical features. They found that all patients, from the beginning had anemia and only 6 children presented with pulmonary symptoms.^[6] In our case the predominant feature was anemia refractory to iron therapy along with chest infiltrates and episodes of hemoptysis. It is difficult to diagnose IPH because nutritional iron deficiency anemia is common in children, as is TB, which may present with lung infiltrates not responding to routinely used antibiotics.^[7] In case of our patient Tuberculosis was ruled out by Sputum AFB and CBNAAT study. The diagnosis of IPH can be confirmed only after excluding other causes of pulmonary hemorrhage, such as mitral stenosis with congestive cardiac failure, Peri Arteritis Nodosa, Wegener's granulomatosis, Good-pasture's

syndrome, Systemic lupus erythematosus, coagulopathies etc.^[4,8] In our patient, investigations such as echocardiography, anti neutrophil cytoplasmic antibody (ANCA), antinuclear antibody (ANA), anti-GBM antibody, and coagulation profile were all found to be negative, thus excluding these common causes of pulmonary bleeding. The gold standard for IPH diagnosis is lung biopsy. On the other hand, diagnosis of IPH can be confirmed by bronchoscopy with broncho alveolar lavage. showing hemosiderin-laden macrophages.^[5] Khonglah Y et al and Bakalli I et al in their respective studies have found siderophages in gastric lavage fluid, which is equally diagnostic and also the simplest, reliable test in infants and young children.^[1,9] Our case was diagnosed by the presence of abundant hemosiderin laden macrophages in the gastric lavage fluid which was confirmed by Perl's Prussian blue stain for hemosiderin. Corticosteroids have a favorable effect in acute episodes of alveolar hemorrhage, although there have been few studies showing that, in patients with IPH, they are protective against recurrence or evolution to pulmonary fibrosis.[10] Low dose immuno suppressants like Azathioprine added with corticosteroids is also used in recent times for therapy.^[10] Our patient on diagnosis was started with parental steroid therapy supported by iron and vitamin supplementation. The patient at present is stable and under follow up.

IV. Conclusion

Idiopathic pulmonary Hemosiderosis is a rare disorder and the diagnosis is by exclusion. Therefore, definitive diagnosis early and aggressive immunosuppressive therapy of idiopathic pulmonary hemosiderosis (IPH) are required to prevent pulmonary fibrosis and mortality in these patients. Late diagnosis may vield poorer prognosis. Obtaining broncho alveolar lavage in neonates and children can be difficult and in centers where bronchoscopy is not available, the present of hemosiderin laden macrophages in the gastric lavage fluid is equally diagnostic and also the simplest, reliable diagnostic test.

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BCG Vaccine Induces Immunity and Protects Against COVID-19?

By Dr. Bhavani. R

Abstract- Introduction: The Current Respiratory tract disease Pandemic, COVID-19, is a new viral pathogen strain of the Coronavirus family called as the Severe Acute Respiratory Syndrome Coronavirus-2 (SARS- CoV-2). The main complication of the disease includes Severe Pneumonia and Acute Respiratory Distress Syndrome (ARDS). The Bacillus – Calmette–Guerin (BCG) is a live attenuated vaccine developed against Tuberculosis (TB) at the beginning of the 20th century at the Institute Pasteur in Paris. BCG vaccination, in general, is reported to decrease human susceptibility to respiratory tract infections (O'Neill & Netea, 2020) and also reduces infant mortality. This protective effect of BCG appears to be common against any unrelated infectious agents and respiratory tract infections. Thus we aimed to study on whether BCG vaccine induces immunity and protects against the new respiratory infectious disease, COVID-19.

Keywords: COVID-19, BCG vaccination, Trained immunity, SARS-CoV-2, Respiratory tract infection.

GJMR-C Classification: NLMC Code: QW 800

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Materials and Method: We searched various databases like PubMed, Medline, and Google scholar for articles explaining the effect of BCG against respiratory disease and COVID-19. The keywords used were "BCG vaccine" AND "COVID- 19" OR "BCG Vaccine against Respiratory Infections" AND "COVID-19 disease".

Results: Our results showed that BCG vaccination induces trained immunity against several viral infections. Some studies consider that BCG vaccination is a protective factor for COVID-19. Because countries without long-standing BCG vaccination policy have been more severely affected with COVID-19 than countries with such policy.

Conclusion: Thus, we conclude that BCG can have protective action against COVID-19, but more clinical trials needed to confirm the hypothesis.

Keywords: COVID-19, BCG vaccination, Trained *immunity*, SARS-CoV-2, Respiratory tract infection.

I. INTRODUCTION

Goronavirus disease is a respiratory infection originated in central China and quickly spread into many countries, which may lead to complications like Severe Pneumonia and Acute Respiratory Distress Syndrome (ARDS) (O'Neill & Netea, 2020). The disease is reported to be caused by a new strand in coronavirus family, namely Severe Acute Respiratory Syndrome Coronavirus-2 (SARS-CoV-2). Coronaviruses (CoVs) belong to the subfamily Orthocoronavirinae in the family Coronaviridae, Order Nidovirales. There are four genera within the subfamily Orthocoronavirus (β-CoV), Gamma coronavirus (γ-CoV)

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and Delta coronavirus (δ -CoV) (Banerjee et al., 2019). The CoV genome is an enveloped, positive-sense, single-stranded RNA with a size varying between 26 kb and 32 kb, the largest genome of known RNA viruses (Banerjee et al., 2019).

Bacillus – Calmette–Guerin (BCG) is a live attenuated vaccine developed against Tuberculosis at the beginning of the 20th century at the Institute Pasteur in Paris. Since then, it is referred to be the most used vaccine in the world, with around 130 million children vaccinated every year. Many randomized control trials have reported 50% reduction of mortality among young infants vaccinated with BCG (Aaby et al., 2011). This reduction appears to be due to the protective action of BCG against unrelated infectious agents, especially respiratory tract infections and neonatal sepsis (O'Neill & Netea, 2020).

COVID-19 has caused large number of deaths with tens of thousands of cases confirmed worldwide, thus posing a serious threat to Global Public Health (Li et al., 2020). However, currently there are no clinically proven vaccines or specific therapeutic drugs available to control or contain the disease spread (Aaby et al., 2011).

Thus, considering the protective action of BCG vaccine against major respiratory infections in general, we aimed to study its effect in inducing immunity against COVID-19.

II. Methods

Various articles reporting the effectiveness of BCG vaccination against respiratory diseases were searched in database like PUBMED, MEDLINE, and GOOGLE SCHOLAR. The keywords used were "BCG vaccine" AND "COVID 19" OR ""BCG Vaccine against Respiratory Infections" AND "COVID-19 disease".

III. Results

a) BCG Induced Trained Immunity against COVID-19

In general, everyone is aware that the BCG vaccine is the most used vaccine worldwide, with around 130 million children vaccinated every year. But many epidemiological studies have reported that the BCG vaccine has reduced the infant mortality globally (O'Neill & Netea, 2020). Everyone well knows that virus pathogen is the most common cause of respiratory tract infection and mortality in children and BCG, an effective

vaccine against TB, with protective action against all respiratory infections, can be concluded as the reason for the reduction in mortality among children affected by respiratory diseases.

A community-based case-control study from Guinea Bissau has showed that BCG vaccine reduces the incidence of respiratory syncytial virus infection (Stensballe et al., 2005). BCG vaccination significantly increases the secretion of pro-inflammatory cytokines, specifically IL-1B and plays a vital role in antiviral immunity (Kleinnijenhuis et al., 2014). A recent randomized, placebo-controlled human challenge study has also showed that BCG vaccination induced genome-wide epigenetic reprogramming of monocytes and protected against an attenuated yellow fever virus vaccine strain (Arts et al., 2018). These study findings thus strengthen the fact that inducing trained immunity by BCG vaccine results in significant protection against several viral infections.

b) Does BCG vaccination policy reduce morbidity and mortality of COVID-19?

BCG, a live attenuated vaccine used worldwide against Tuberculosis including many nations like Japan and China, have a universal BCG vaccination policy among newborns (*Correlation between Universal BCG Vaccination Policy and Reduced Morbidity and Mortality* for COVID-19: An Epidemiological Study | MedRxiv, n.d.a). A recent epidemiological study showed that the countries without long-standing BCG policy had been more severely affected with COVID -19 than countries with such policy (Correlation between Universal BCG Vaccination Policy and Reduced Morbidity and Mortality for COVID-19: An Epidemiological Study | MedRxiv, n.d.-a).

They classified the countries into three categories low-income, low-middle income, and high-income based on GNI per capita in 2018. The study reported that the low-income countries showed less number of deaths attributed to COVID-19 due to long-standing BCG policy, than low-middle income and high-income countries (Italy, Belgium, Netherlands, United States, and Lebanon). In Italy with no universal vaccination policy, the COVID-19 case mortality is very high in spite of implementing preventive measures like social distancing and strict Isolation.

On the other hand, countries like Japan with no strict isolation measures, managed to maintain reduced mortality rate. This may be due to their long-standing BCG policy and this can be considered as an evidence that the BCG vaccination policy can be a protective factor against COVID-19 infection.



Figure 1: Numbers of COVID 19 cases in countries that never implemented a universal BCG vaccination policy(*Correlation between Universal BCG Vaccination Policy and Reduced Morbidity and Mortality for COVID-19: An Epidemiological Study* | *MedRxiv*, n.d.-b).



Figure 2: Higher death rate in countries that never implemented a universal BCG vaccination policy(*Correlation between Universal BCG Vaccination Policy and Reduced Morbidity and Mortality for COVID-19: An Epidemiological Study* | *MedRxiv*, n.d.-b).

The recent ecological study examined the effects of BCG vaccination on countries affected with COVID-19, based on cases and deaths of people due to the disease and exponential growth factors over specific periods of the Pandemic (Sala et al., 2020). The results of the study suggested that BCG vaccination and exposure to tuberculosis may induce non-specific protection against the novel SARS-CoV-2 infection. However, these findings can neither be hastily dismissed nor taken as ultimate evidence and this brings up the need more clinical trial to confirm the hypothesis.

c) Confounding factors

Most studies though showed supportive evidences for the hypothesis that BCG has protective action against COVID-19, were still affected by potential confounding factors like –the different phases of the outbreak, mean age of the affected population, management of the pandemic, administration of number of tests, definitions of COVID-19–related deaths, or underreporting.

A recent cohort study in Israeli tried to remove these confounding factors. In Israeli, between the period of 1955 to 1982, BCG vaccinations were included as a national immunization program and covered 90% of population. After 1982, the use of vaccine was restricted to be administered only to immigrants from countries with a high prevalence of Tuberculosis (Hamiel et al., 2020a). This change allowed comparison of infection rates (Table 1) and proportions with severe COVID-19 disease in 2 similar populations with differing BCG status-Individuals born during the three years before and three years after cessation of the universal BCG vaccine programme (Hamiel et al., 2020a).

	Birth	n Year	Difference	P-value	
	1979-1981 (BCG vaccinated)	1983-1985 (BCG unvaccinated)	(95% Cl)		
Total population	297340	301600			
Immigrants in total population, No. (%) ^a	14 569 (4.9)	13 873 (4.6)			
No. of test	3064	2869			
Proportion of population tested, %	1.02	0.96			

Table 1: Results of SARS-CoV-2 PCR Testing by Age Group(Hamiel et al., 2020b)

Men tested, No. (%)	1509 (49.2)	1458 (50.8)		.29
Positive results				
No. (%)	361 (11.7)	299 (10.4)	1.3 (-0.3 to 2.9)	.09
No. per 100 000 population in age group ^b	121	100	21 (-10 to 50)	.15
Men with positive result, No (%)	181 (50)	152 (51)		.87
No. with severe disease	1	1		

Abbreviations: PCR, polymerase chain reaction; SARS-CoV-2, severe acute respiratory syndrome coronavirus 2.

- Number of immigrants from countries with BCG vaccination policy and in their total population included in different birth-year groups.
- Rates per 100 000 population do not represent the positivity rate in the population because the persons

The result of the study showed that there was no statistically significant difference in the proportion of positive test results in the BCG-vaccinated group (361 [11.7%]) vs the unvaccinated group (299 [10.4%]; difference, 1.3%; 95%CH,0.3%to 2.9%; P = .09) (Hamiel et al., 2020a).

Thus, the study does not conclude any supportive evidence to accept the hypothesis that BCG vaccination in childhood has a protective effect against COVID 19 in childhood. The strength of the study is a large population-based cohort and the comparison of two similar groups with limiting the confounding to the minimum (Hamiel et al., 2020a).

IV. DISCUSSION

SARS-CoV first appeared in 2002 and rapidly spread to 32 countries and regions, after which the world experienced the then MERS-CoV outbreak in 2012(Li et al., 2020). SARS-CoV-2 has spread rapidly in multiple countries causing severe illness, and sustained human-to-human transmission, making it a serious public-health threat to be concerned (Li et al., 2020). The disease since its first outbreak, has been a major challenge for public health authorities to control and there is currently no officially approved vaccine to protect from the disease.

Many ecological studies have tried to prove that BCG vaccination induces immunity and protection against COVID-19. But these studies are affected by bias from various confounding factors such as differences in national demographics and disease burden, testing rates for COVID-19 virus infections, and the stage of the pandemic in each country. Some cohort studies tried to limit these confounders and conclude with supportive evidences, still failed. The studies aimed to state that countries with BCG vaccination policy showing reduced morbidity and mortality makes the vaccine, a potential new tool to fight against COVID-19. But without doing any clinical trial, we can neither accept nor eliminate this hypothesis.

WHO reported that there is no evidence that the Bacillus – Calmette-Guérin vaccine (BCG) protects the people fromCOVID-19 infection. This lead to two clinical trials to address this question which are underway, and the WHO will evaluate the evidence when it is available (*Bacille Calmette-Guérin (BCG) Vaccination and COVID-19*, n.d.).

Thus, in the absence of evidence, BCG is not recommended as a prevention against COVID-19 (*Bacille Calmette-Guérin (BCG) Vaccination and COVID-19*, n.d.). In the meantime, the factual theory of protective effect of BCG against respiratory infections in general can be considered as a supportive evidence in its conclusion.

V. Conclusion

Thus, as the whole world is looking for preventive vaccination for COVID-19, any study findings that prove that BCG vaccination induces immunity and protection against COVID-19 cannot be carelessly eliminated. However, they can neither be taken as ultimate supportive evidence, hence raising the need for more clinical trials to confirm the hypothesis.

Compliance with Ethical Standards

Conflict of interest: The authors have no competing interests to declare.

Ethical approval and informed consent: Not applicable

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Certificate, LOR and Laser-Momento

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We accept the manuscript submissions in any standard (generic) format.

We typeset manuscripts using advanced typesetting tools like Adobe In Design, CorelDraw, TeXnicCenter, and TeXStudio. We usually recommend authors submit their research using any standard format they are comfortable with, and let Global Journals do the rest.

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Acknowledgments

Contributors to the research other than authors credited should be mentioned in Acknowledgments. The source of funding for the research can be included. Suppliers of resources may be mentioned along with their addresses.

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Preparing your Manuscript

Authors can submit papers and articles in an acceptable file format: MS Word (doc, docx), LaTeX (.tex, .zip or .rar including all of your files), Adobe PDF (.pdf), rich text format (.rtf), simple text document (.txt), Open Document Text (.odt), and Apple Pages (.pages). Our professional layout editors will format the entire paper according to our official guidelines. This is one of the highlights of publishing with Global Journals—authors should not be concerned about the formatting of their paper. Global Journals accepts articles and manuscripts in every major language, be it Spanish, Chinese, Japanese, Portuguese, Russian, French, German, Dutch, Italian, Greek, or any other national language, but the title, subtitle, and abstract should be in English. This will facilitate indexing and the pre-peer review process.

The following is the official style and template developed for publication of a research paper. Authors are not required to follow this style during the submission of the paper. It is just for reference purposes.

Manuscript Style Instruction (Optional)

- Microsoft Word Document Setting Instructions.
- Font type of all text should be Swis721 Lt BT.
- Page size: 8.27" x 11¹", left margin: 0.65, right margin: 0.65, bottom margin: 0.75.
- Paper title should be in one column of font size 24.
- Author name in font size of 11 in one column.
- Abstract: font size 9 with the word "Abstract" in bold italics.
- Main text: font size 10 with two justified columns.
- Two columns with equal column width of 3.38 and spacing of 0.2.
- First character must be three lines drop-capped.
- The paragraph before spacing of 1 pt and after of 0 pt.
- Line spacing of 1 pt.
- Large images must be in one column.
- The names of first main headings (Heading 1) must be in Roman font, capital letters, and font size of 10.
- The names of second main headings (Heading 2) must not include numbers and must be in italics with a font size of 10.

Structure and Format of Manuscript

The recommended size of an original research paper is under 15,000 words and review papers under 7,000 words. Research articles should be less than 10,000 words. Research papers are usually longer than review papers. Review papers are reports of significant research (typically less than 7,000 words, including tables, figures, and references)

A research paper must include:

- a) A title which should be relevant to the theme of the paper.
- b) A summary, known as an abstract (less than 150 words), containing the major results and conclusions.
- c) Up to 10 keywords that precisely identify the paper's subject, purpose, and focus.
- d) An introduction, giving fundamental background objectives.
- 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.
- f) Results which should be presented concisely by well-designed tables and figures.
- g) Suitable statistical data should also be given.
- h) All data must have been gathered with attention to numerical detail in the planning stage.

Design has been recognized to be essential to experiments for a considerable time, and the editor has decided that any paper that appears not to have adequate numerical treatments of the data will be returned unrefereed.

- i) Discussion should cover implications and consequences and not just recapitulate the results; conclusions should also be summarized.
- j) There should be brief acknowledgments.
- k) There ought to be references in the conventional format. Global Journals recommends APA format.

Authors should carefully consider the preparation of papers to ensure that they communicate effectively. Papers are much more likely to be accepted if they are carefully designed and laid out, contain few or no errors, are summarizing, and follow instructions. They will also be published with much fewer delays than those that require much technical and editorial correction.

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



Format Structure

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

All manuscripts submitted to Global Journals should include:

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The title page must carry an informative 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) where the work was carried out.

Author details

The full postal address of any related author(s) must be specified.

Abstract

The abstract is the foundation of the research paper. It should be clear and concise and must contain the objective of the paper and inferences drawn. It is advised to not include big mathematical equations or complicated jargon.

Many researchers searching for information online will use search engines such as Google, Yahoo or others. By optimizing your paper for search engines, you will amplify the chance of someone finding it. In turn, this will make it more likely to be viewed and cited in further works. Global Journals has compiled these guidelines to facilitate you to maximize the web-friendliness of the most public part of your paper.

Keywords

A major lynchpin of research work for the writing of research papers is the keyword search, which one will employ to find both library and internet resources. Up to eleven keywords or very brief phrases have to be given to help data retrieval, mining, and indexing.

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

Choice of the main keywords is the first tool of writing a research paper. Research paper writing is an art. Keyword search should be as strategic as possible.

One should start brainstorming lists of potential keywords before even beginning 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 a research paper?" Then consider synonyms for the important words.

It may take the discovery of only one important paper to steer in the right keyword direction because, in most databases, the keywords under which a research paper is abstracted are listed with the paper.

Numerical Methods

Numerical methods used should be transparent and, where appropriate, supported by references.

Abbreviations

Authors must list all the abbreviations used in the paper at the end of the paper or in a separate table before using them.

Formulas and equations

Authors are advised to submit any mathematical equation using either MathJax, KaTeX, or LaTeX, or in a very high-quality image.

Tables, Figures, and Figure Legends

Tables: Tables should be 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. Authors must submit tables in an editable format and not as images. References to these tables (if any) must be mentioned accurately.

Figures

Figures are supposed to be submitted as separate files. Always include a citation in the text for each figure using Arabic numbers, e.g., Fig. 4. Artwork must be submitted online in vector electronic form or by emailing it.

Preparation of Eletronic Figures for Publication

Although low-quality images are sufficient for review purposes, print publication requires high-quality images to prevent the final product being blurred or fuzzy. Submit (possibly by e-mail) EPS (line art) or TIFF (halftone/ photographs) files only. MS PowerPoint and Word Graphics are unsuitable for printed pictures. Avoid using pixel-oriented software. Scans (TIFF only) should have a resolution of at least 350 dpi (halftone) or 700 to 1100 dpi (line drawings). 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).

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TIPS FOR WRITING A GOOD QUALITY MEDICAL RESEARCH PAPER

1. *Choosing the topic:* In most cases, the topic is selected by the interests of the author, but it can also be suggested by the guides. You can have several topics, and then judge which you are most comfortable with. This may be done by asking several questions of yourself, like "Will I be able to carry out a search in this area? Will I find all necessary resources to accomplish the search? Will I be able to find all information in this field area?" If the answer to this type of question is "yes," then you ought to choose that topic. In most cases, you may have to conduct surveys and visit several places. Also, you might have to do a lot of work to find all the rises and falls of the various data on that subject. Sometimes, detailed information plays a vital role, instead of short information. Evaluators are human: The first thing to remember is that evaluators are also human beings. They are not only meant for rejecting a paper. They are here to evaluate your paper. So present your best aspect.

2. *Think like evaluators:* If you are in confusion or getting demotivated because your paper may not be accepted by the evaluators, then think, and try to evaluate your paper like an evaluator. Try to understand what an evaluator wants in your research paper, and you will automatically have your answer. 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.

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

4. Use of computer is recommended: As you are doing research in the field of medical research then this point is quite obvious. Use right software: Always use good quality software packages. If you are not capable of judging good software, then you can lose the quality of your paper unknowingly. There are various programs available to help you which you can get through the internet.

5. Use the internet for help: An excellent start for your paper is using Google. It is a wondrous search engine, where you can have your doubts resolved. You may also read some answers for the frequent question of how to write your research paper or find a model research paper. You can download books from the internet. If you have all the required books, place importance on reading, selecting, and analyzing the specified information. Then sketch out your research paper. Use big pictures: You may use encyclopedias like Wikipedia to get pictures with the best resolution. At Global Journals, you should strictly follow here.

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

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

8. *Make every effort:* Make every effort to mention what you are going to write in your paper. That means always have a good start. Try to mention everything in the introduction—what is the need for a particular research paper. Polish your work with good writing skills and always give an evaluator what he wants. Make backups: When you are going to do any important thing like making a research paper, you should always have backup copies of it either on your computer or on paper. This protects you from losing any portion of your important data.

9. Produce good diagrams of your own: Always try to include good charts or diagrams in your paper to improve quality. Using several unnecessary diagrams will degrade the quality of your paper by creating a hodgepodge. So always try to include diagrams which were made by you to improve the readability of your paper. Use of direct quotes: When you do research relevant to literature, history, or current affairs, then use of quotes becomes essential, but if the study is relevant to science, use of quotes is not preferable.

10. Use proper verb tense: Use proper verb tenses in your paper. Use past tense to present those events that have happened. Use present tense to indicate events that are going on. Use future tense to indicate events that will happen in the future. Use of wrong tenses will confuse the evaluator. Avoid sentences that are incomplete.

11. Pick a good study spot: Always try to pick a spot for your research which is quiet. Not every spot is good for studying.

12. *Know what you know:* Always try to know what you know by making objectives, otherwise you will be confused and unable to achieve your target.

13. Use good grammar: Always use good grammar and words that will have a positive impact on the evaluator; use of good vocabulary does not mean using tough words which the evaluator has to find in a dictionary. Do not fragment sentences. Eliminate one-word sentences. Do not ever use a big word when a smaller one would suffice.

Verbs have to be in agreement with their subjects. In a research paper, do not start sentences with conjunctions or finish them with prepositions. When writing formally, it is advisable to never split an infinitive because someone will (wrongly) complain. Avoid clichés like a disease. Always shun irritating alliteration. Use language which is simple and straightforward. Put together a neat summary.

14. 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 for your topic. You may also maintain your arguments with records.

15. Never start at the last minute: Always allow enough time for research work. Leaving everything to the last minute will degrade your paper and spoil your work.

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

17. *Never copy others' work:* Never copy others' work and give it your name because if the evaluator has seen it anywhere, you will be in trouble. Take proper rest and food: No matter how many hours you spend on your research activity, if you are not taking care of your health, then all your efforts will have been in vain. For quality research, take proper rest and food.

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

19. Refresh your mind after intervals: Try to give your mind a rest by listening to soft music or sleeping in intervals. This will also improve your memory. Acquire colleagues: Always try to acquire colleagues. No matter how sharp you are, if you acquire colleagues, they can give you ideas which will be helpful to your research.

20. *Think technically:* Always think technically. If anything happens, search for its reasons, benefits, and demerits. Think and then print: When you go to print your paper, check that tables are not split, headings are not detached from their descriptions, and page sequence is maintained.

21. Adding unnecessary information: Do not add unnecessary information like "I have used MS Excel to draw graphs." Irrelevant and inappropriate material is superfluous. Foreign terminology and phrases are not apropos. One should never take a broad view. Analogy is like feathers on a snake. Use words properly, regardless of how others use them. Remove quotations. Puns are for kids, not grunt readers. Never oversimplify: When adding material to your research paper, never go for oversimplification; this will definitely irritate the evaluator. Be specific. Never use rhythmic redundancies. Contractions shouldn't be used in a research paper. Comparisons are as terrible as clichés. Give up ampersands, abbreviations, and so on. Remove commas that are not necessary. Parenthetical words should be between brackets or commas. Understatement is always the best way to put forward earth-shaking thoughts. Give a detailed literary review.

22. Report concluded results: Use concluded results. From raw data, filter the results, and then conclude your studies based on measurements and observations taken. An appropriate number of decimal places should be used. Parenthetical remarks are prohibited here. Proofread carefully at the final stage. At the end, give an outline to your arguments. Spot perspectives of further study of the subject. Justify your conclusion at the bottom sufficiently, which will probably include examples.

23. Upon 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 for 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 of 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 criteria peer reviewers will use for grading the final paper.

Final points:

One purpose of organizing a research paper is to let people interpret your efforts selectively. The journal requires the following sections, submitted in the order listed, with each section starting on a new page:

The introduction: This will be compiled from reference matter and reflect the design processes or outline of basis that directed you to make a study. As you carry out the process of study, the method and process section will be constructed like that. The results segment will show related statistics in nearly sequential order and direct reviewers to similar intellectual paths throughout the data that you gathered to carry out your study.

The discussion section:

This will provide understanding of the data and projections as to the implications of the results. The use of good quality references throughout the paper will give the effort trustworthiness by representing an alertness to 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 progression.

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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.



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- Separating a table, chart, or figure—confine each to a single page.
- Submitting a manuscript with pages out of sequence.
- In every section of your document, use standard writing style, including articles ("a" and "the").
- Keep paying attention to the topic of the paper.
- Use paragraphs to split each significant point (excluding the abstract).
- Align the primary line of each section.
- Present your points in sound order.
- Use present tense to report well-accepted matters.
- Use past tense to describe specific results.
- Do not use familiar wording; don't address the reviewer directly. Don't use slang or superlatives.
- Avoid use of extra pictures—include only those figures essential to presenting results.

Title page:

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

Abstract: This summary should be two hundred words or less. It should clearly and briefly explain the key findings reported in the manuscript and must have precise statistics. It should not have acronyms or abbreviations. It should be logical in itself. Do not cite 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 approaches 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. Use comprehensive sentences, and do not sacrifice readability for brevity; you can maintain it succinctly by phrasing sentences so that they provide more than a 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 limit the initial two items to no more than one line each.

Reason for writing the article—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 for this; results of any numerical analysis should be reported. Significant conclusions or questions that emerge from the research.

Approach:

- Single section and succinct.
- An outline of the job done is always written in past tense.
- o Concentrate on shortening results—limit background information to a verdict or two.
- Exact spelling, clarity 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 of comprehending and calculating the purpose of your study without having to refer to other works. The basis for the study should be offered. Give the most important references, but avoid making a comprehensive appraisal of the topic. Describe the problem visibly. If the problem is not acknowledged in a logical, reasonable way, the reviewer will give no attention to your results. Speak in common terms about techniques used to explain the problem, if needed, but do not present any particulars about the protocols here.

The following approach can create a valuable beginning:

- Explain the value (significance) of the study.
- Defend the model—why did you employ this particular system or method? What is its compensation? Remark upon its appropriateness from an abstract point of view as well as pointing out sensible reasons for using it.
- Present a justification. State your particular theory(-ies) or aim(s), and describe the logic that led you to choose them.
- o Briefly explain the study's tentative purpose and how it meets 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 for every section. If you make the four points listed above, you will need at least four paragraphs. Present surrounding information only when it is necessary to support a situation. The reviewer does not desire to read everything you know about a topic. Shape the theory 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 soundly written procedures segment allows a capable scientist to replicate 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 to give the least amount of information that would permit another capable scientist to replicate 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 section, mention the specific item describing the way, but draw the basic principle while stating the situation. The purpose is to show 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:

Materials may be reported in part of a section or else they may be recognized along with your measures.

Methods:

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

Approach:

It is embarrassing to use vigorous voice when documenting methods without using first person, which would focus the reviewer's interest on the researcher rather than the job. As a result, when writing up the methods, most authors use third person passive voice.

Use standard style in this and 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.
- o Skip all descriptive information and surroundings—save it for the argument.
- Leave out information that is immaterial to a third party.

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Results:

The principle of a results segment is to present and demonstrate your conclusion. Create this part as 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. Use statistics and tables, if suitable, to present consequences most efficiently.

You must clearly differentiate material which would usually be incorporated in a study editorial from any unprocessed data or additional appendix matter that would not be available. In fact, such matters should not be submitted at all except if requested by the instructor.

Content:

- o Sum up your conclusions in text and demonstrate them, if suitable, with figures and tables.
- o In the manuscript, explain each of your consequences, and point the reader to remarks that are most appropriate.
- Present a background, such as by describing the question that was addressed by creation of an exacting study.
- Explain results of control experiments and give 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 manuscript.

What to stay away from:

- o Do not discuss or infer your outcome, report surrounding information, or try to explain anything.
- Do not include raw data or intermediate calculations in a research manuscript.
- o Do not present similar data more than once.
- o A manuscript should complement any figures or tables, not duplicate information.
- Never confuse figures with tables—there is a difference.

Approach:

As always, use past tense when you submit 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 section.

Figures and tables:

If you put figures and tables at the end of some details, make certain that they are visibly distinguished from any attached appendix materials, such as raw facts. Whatever the position, each table must be titled, numbered one after the other, and include a heading. All figures and tables must be divided from the text.

Discussion:

The discussion is expected to be the trickiest segment to write. A lot of papers submitted to the journal are discarded based on problems with the discussion. There is no rule for how long an 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 implications of the study. The purpose here is to offer an understanding of your results and support all of your conclusions, using facts from your research and generally accepted information, if suitable. The implication of results should be fully 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 the prospect, and let it drop at that. Make a decision as to whether each premise is supported or 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 an experiment might be personalized to accomplish a new idea.
- Give details of all of your remarks as much as possible, focusing on mechanisms.
- Make a decision as to whether the tentative design sufficiently addressed the theory and whether or not it was correctly restricted. Try to present substitute explanations if they are sensible alternatives.
- One piece of 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?
- o Recommendations for detailed papers will offer supplementary suggestions.

Approach:

When you refer to information, differentiate data generated by your own studies from other available information. Present work done by specific persons (including you) in past tense.

Describe generally acknowledged facts and main beliefs in present tense.

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Introduction	Containing all background details with clear goal and appropriate details, flow specification, no grammar and spelling mistake, well organized sentence and paragraph, reference cited	Unclear and confusing data, appropriate format, grammar and spelling errors with unorganized matter	Out of place depth and content, hazy format
Methods and Procedures	Clear and to the point with well arranged paragraph, precision and accuracy of facts and figures, well organized subheads	Difficult to comprehend with embarrassed text, too much explanation but completed	Incorrect and unorganized structure with hazy meaning
Result	Well organized, Clear and specific, Correct units with precision, correct data, well structuring of paragraph, no grammar and spelling mistake	Complete and embarrassed text, difficult to comprehend	Irregular format with wrong facts and figures
Discussion	Well organized, meaningful specification, sound conclusion, logical and concise explanation, highly structured paragraph reference cited	Wordy, unclear conclusion, spurious	Conclusion is not cited, unorganized, difficult to comprehend
References	Complete and correct format, well organized	Beside the point, Incomplete	Wrong format and structuring

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