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Editorial

NOVEL CORONAVIRUS (NCOV-2019): A GLOBAL HEALTH EMERGENCY

Tanzeela Zafar

Globally, public health institutes are on high alert after the recent declaration of an epidemic of Novel coronavirus in China, by the World health organization. nCoV-2019 has been labeled as a **public health emergency of international concern** (PHEIC) by WHO.[1] In mid-December 2019, several cases of pneumonia-like disease had been reported in the central Chinese city of Wuhan. Chinese health authorities conducted immediate investigations of those clustered cases to identify the causative agent of that disease and to halt its spread. Later, Chinese scientists isolated Novel Coronavirus from the identified patients in early January 2020.[2] Till 12th February 2020, more than 45,000 cases infected with nCoV-2019 have been reported. The death toll to the date is 1115.[3] Novel coronavirus was first identified in China but later it was introduced outside China by infected travelers. The first international case outside China was reported in Thailand. Since then, nCoV-2019 has been reported in 28 other countries around the world.[4]

Coronavirus is a large family of viruses, known for causing potentially deadly diseases in mammals and birds. The name "coronavirus" originated from the Latin word 'corona' meaning crown or halo, which in turn reflects the characteristic appearance of the virus particle.[5] The earliest coronaviruses were discovered in the 1960s, from nasal cavities of human patients, who presented with common cold. Later those viruses were named as human coronavirus 229E and human Coronavirus OC43.[6] Since then, the other members of this virus family identified include SARS (2003), HCoV NL63 (2004), MERS-CoV (2012).[7,8]

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Later, in the same decade the discovery of this Novel coronavirus has attracted by far the most attention. On 11th February 2020, the World Health Organization has announced an official name "COVID-19" for the disease caused by nCoV-2019.[9]

Coronavirus causes significant percentage of all common colds in humans and is transmitted in a similar fashion from person-to-person via respiratory droplets.[10] The most common presentation is fever, cough and shortness of breath. The spectrum of disease ranged from mild or no symptoms to either severe respiratory illness and death. Case-fatality rate for 2019-nCoV is estimated to be 2.3%. The attack rate and transmissibility of virus is relatively high.[11]

No specific antiviral treatment has yet recommended by WHO for 2019-nCoV. However, isolation and symptomatic care are indicated for an infected person. For those presenting with severe illness, treatment modalities should include intensive care and monitoring.[12]

No specific vaccine is currently available for 2019-nCoV. The best strategy for prevention and control, as recommended by CDC, is to avoid exposure to this virus. The infection control measures include administrative rules, engineering controls, correct work practices, appropriate usage of personal protective equipment. Prompt patient detection, effective triage, isolation of potentially infectious patients and two weeks quarantine of the contacts are essential steps too, to prevent unnecessary exposure.[13] The cautious approach must also include other general measures like frequent washing of hands with water and soap, avoid touching nose, mouth, and eyes with unwashed hands, avoid close contact with the sick person, and cough etiquette.[14] WHO also emphasizes upon screening precautions for ongoing travelers.[15]

The outbreak of 2019-nCoV and the evidence of its likely person-to-person transmission highlights the importance of vigilant and rapid investigation of cases and high-risk contacts. To contract this deadly disease incidence, prevalence, and mortality figures, strategies for mass-awareness should be formulated to help people understand the nature of the disease and the relative preventive measures. This will also help to reduce the associated panic created in the general public. The aim of those awareness programs should also include encouraging people to report to their healthcare providers immediately if they develop signs/symptoms of respiratory illness within 14 days of their travel from China or had close contact with someone who had recently traveled from China.

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Original Article:

IMPACT OF EDUCATION ON PRACTICES OF ANTENATAL CARE IN PREGNANT WOMEN: AN ANALYTICAL SURVEY

Kamran Ashraf[1], Iram Manzoor[2], Abida Hassan[3], Qurat ul Ain Zulfi[4], Minahil Iqbal[5].

ABSTRACT:

Background and Objective: Maternal health is a global public health challenge that is directly affected by the practices during the antenatal period. The objective of this study was to determine factors influencing the effective utilization of antenatal services and to assess the impact of education of women toward practices of antenatal care in women of the reproductive age group in Lahore.

Material and Methods: An analytical cross-sectional study was conducted at Lady Willingdon Hospital, Farooq Hospital, and Akhter Saeed Trust Teaching Hospital, Lahore from March to August 2019. This study included 262 pregnant mothers selected by non-probability convenience sampling from three tertiary care hospitals of Lahore. Data was collected on a structured questionnaire and was analyzed on SPSS version 22. Chi-square test was applied to assess the impact of education on the effective utilization of antenatal services and practices followed during this period.

Results: Out of 262 pregnant women, who participated in this research 199(75.97%) were literate. The majority of them; 196(74.80%) were multigravida. Only 145(55.3%) had planned pregnancy regarding antenatal practices, 66% had reported intake of folic acid, 69.8% iron supplements, and 71.8% calcium supplements. Only 58.8% had TT vaccination coverage. On bivariate analysis, it was observed that strong association was seen between educational status and time of reporting pregnancy (p=0.001), planned pregnancy (p=0.058), folic acid supplementation (p=0.000) iron supplementation (p=0.000), calcium supplementation (p=0.046), hospital delivery (p=0.03) and delivery by a doctor (p=0.024).

Conclusion: Education has a positive impact on good practices during the antenatal period.

Key Words: Antenatal care practices, Multivitamin Supplementation, Planned Pregnancy,

INTRODUCTION:

Antenatal care (ANC) is the care provided to all pregnant women to ensure the best health conditions for the women and their fetuses during pregnancy.[1] WHO recommends a goal-oriented approach that is the diagnosis of pregnancy and the utilization of antenatal services should be as early as possible. WHO recommends a minimum of four antenatal visits for all pregnant mothers, however, statistics show less than four visits, in many developing countries. On a global scale, it is estimated that approximately 80% of maternal deaths[2] and up to two-thirds of neonatal deaths could be avoided if effective health services are provided during birth and the first week of the life of neonate.[3] Early and regular antenatal service utilization throughout pregnancy is recommended globally to improve health statistics related to pregnancy and its outcome.[4] It has been observed that expectant women in poor socio-economic settings start late service utilization[5] and do not fulfill WHO-defined minimum four antenatal visits.[6] According to a survey done in 2015, the Maternal Mortality Rate in Nepal was estimated to be 229 per100,000 live births, and this indicator constitutes 11% of all deaths in females of 15-49 years.[7] Currently, promotion of maternal health service utilization is on the way, however poor socioeconomic status, rural inhabitation and belonging from ethnic minorities are

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limitations.[8] A qualitative study conducted in Nepal showed that pregnant women's heavy work-load, mother-in-law's perception of benefit of antenatal care, her power, and control over resources, and the relationship between mother-in-law and pregnant women also played a vital role in pregnant women's utilization of antenatal care.[9] Another study suggested that women from rich families have three times more chances of having 4 antenatal care visits compared to women from a poor family.[10] The findings from Pakistan are consistent with analysis from the other low and middle-income countries which suggests that there are substantial gaps between antenatal care coverage and the receipt of WHO-recommended content of care.[11] According to a survey, in Pakistan, antenatal utilization has been increased from the last two decades with the percentage increasing from 26% in 1990-91 to 78% in 2012-13.[12] This study aimed to assess the impact of education in the utilization of antenatal care services in Lahore.

MATERIAL AND METHODS:

An analytical cross-sectional study was conducted in three tertiary care teaching hospitals of district Lahore, all in urban settings. These include one Government-owned hospital; Lady Willingdon Hospital and two in the private sector; Akhter Saeed Trust Teaching Hospital and Farooq Hospital, West Wood, Lahore.

This research was done in Obstetrics and Gynaecology outdoors (OPD) of all three hospitals from March 2019 to August 2019. A total of 262 pregnant mothers fulfilling inclusion criteria and willing to participate were selected through a non-probability convenient sampling method. Consent was taken first from the IRB committee of Akhter Saeed Medical and Dental College and then Medical Superintendent (MS) of concerned hospitals to collect data. The data was collected through a pretested structured questionnaire. The response rate was 100%. Data was collected on gravidity, planned pregnancy, time of antenatal visits and intake of supplements, investigations, and developing complications.

Data was entered and analyzed on SPSS version 22. Chi-square test was applied for bivariate analysis between education and practices during the antenatal period. p-value was fixed at ≤ 0.05 to declare a significant association between two variables.

RESULTS:

This study is conducted on a sample of 262 pregnant females who reported in the OPD of selected hospitals. The socio-demographic profile showed that a vast majority of 220(83.90%) were between 20 – 40 years. As Lahore is an urban and well-developed city, 199(75.95%) of the pregnant females who participated in the study were literate. Out of 262 participants, 250 (95.42%) were housewives and had a monthly income of more than Rs. 10,000. Seventy-four percent of the respondents were multigravida. Only 145(55.3%) had planned their current pregnancy.

Table 1: Socio-demographic profile of Participants

Variables	Frequency n = 262	Percentage (%)
Age		
Less than 20 years	42	16.03
21 – 40 years	220	83.96
Educational Status		
Illiterate	63	24.04
Literate	199	75.95
Employment Status		
Employed	12	4.58
Housewives	250	95.42
Gravidity		
Primi-Gravida	66	25.2
Multi-Gravida	196	74.80
Planned pregnancy		
Yes	145	55.3
No	117	44.7

The results showed poor reporting during early pregnancy. Only 61(23.3%) of the participants reported their pregnancy within first 4 weeks of conception and 201(76.71%) reported it after 4 weeks. Routine tests were conducted diligently by the participants as 236(90%) reported that

they have undergone for complete urine examination, 233(88.9%) had blood examination and 233(88.9%) had done their ultrasounds.

Table 2: Practices during antenatal period

Practices Pregnancy	Frequency n = 262	Percentage (%)
Folic Acid Supplements		
Yes	173	66
No	89	34
Iron Supplements		
Yes	183	69.8
No	79	30.2
Calcium Supplements		
Yes	188	71.8
No	74	28.2
TT Vaccination		
Yes	154	58.8
No	108	41.2

One hundred and seventy-three (66%) expectant mothers reported intake of folic acid. 183(69.8%) iron supplements and 188 (71.8%) calcium supplements. One hundred and fifty-four (58.8%) had tetanus toxoid vaccination. Hypertension and Diabetes Mellitus, two major non-communicable diseases were reported as 55(21%) and 34(12.9%) by the respondents. Hundred pregnant women which constituted (38.2%) were anemic.

Chi-square test was applied to bivariate analysis to assess the effect of education on practices of pregnant women during the current pregnancy. It was observed that there was a significant association between educational status and time of reporting their pregnancy during the first antenatal visit (p=0.001).

Educated mothers had planned pregnancies (p=0.058). There was a significant association between the education of mothers and the intake of folic acid during the first trimester (0.000), intake of iron supplements (0.000), and calcium supplements (0.046). No significant difference was observed for TT vaccine coverage between educated and non-educated women (0.551). Educated women preferred hospital-based deliveries with a p-value of 0.003 as compared to non-educated women, who preferred home-based deliveries. A significant difference was also observed in choices for conduction of deliveries where the majority of educated women preferred doctors for this purpose (p=0.024).

Table 3: Impact of education on practices of antenatal care

Variables	Education		Total	p-value
	Educated	Non-educated		
Antenatal Visits				
1-4 weeks	46 (75.4%)	15 (24.6%)	61 (100.0%)	.001*
5-8 weeks	73 (85.9%)	12 (14.1%)	85 (100.0%)	
9-12 weeks	50 (80.6%)	12 (19.4%)	62 (100.0%)	
13-16 weeks	18 (64.3%)	10 (35.7%)	28 (100.0%)	
17-20 weeks	4 (50.0%)	4 (50.0%)	8 (100.0%)	
More than 20 weeks	8 (44.4%)	10 (55.6%)	18 (100.0%)	
Pregnancy planned				
Yes	116 (80.0%)	29 (20.0%)	145 (100.0%)	.058*
No	83 (70.9%)	34 (29.1%)	117 (100.0%)	
Folate supplements during 1st trimester				
Yes	145 (83.8%)	28 (16.2%)	173 (100.0%)	.000*
No	54 (60.7%)	35 (39.3%)	89 (100.0%)	
Iron supplements during Pregnancy				
Yes	152 (83.1%)	31 (16.9%)	183 (100.0%)	.000*
No	47 (59.5%)	32 (40.5%)	79 (100.0%)	
Calcium supplements pregnancy				
Yes	149 (79.3%)	39 (20.7%)	188 (100.0%)	.046*
No	50 (67.6%)	24 (32.4%)	74 (100.0%)	
TT vaccine during this pregnancy				
Yes	119 (77.3%)	35 (22.7%)	154 (100.0%)	.551
No	80 (74.1%)	28 (25.9%)	108 (100.0%)	
Delivery will be carried out in				
Home	2 (28.6%)	5 (71.4%)	7 (100.0%)	.003*
Hospital	197 (77.3%)	58 (22.7%)	255 (100.0%)	
Delivery will be conducted by				
Doctor	195 (77.4%)	57 (22.6%)	252 (100.0%)	.024*
Dai	3 (42.9%)	4 (57.1%)	7 (100.0%)	
LHV	1 (33.3%)	2 (66.7%)	3 (100.0%)	

*p<0.05 significant

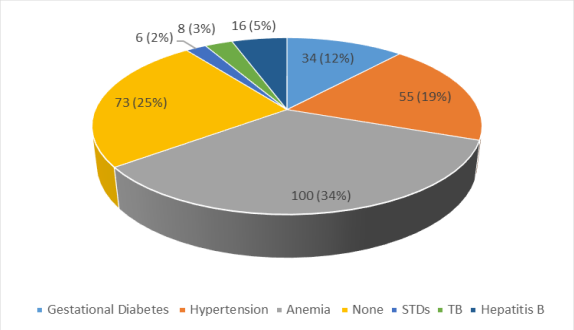

Fig. 1: Comorbidities associated with pregnancy

Results showed that a vast majority of 100(34%) had anemia during this pregnancy. 55(21%) developed hypertension, 34(13%) developed gestational diabetes. A small proportion developed STDs and tuberculosis. Only 28% were reported without any comorbidity.

DISCUSSION:

Antenatal care is a preventive approach to promote and safeguard maternal health as it filters high-risk mothers for further referrals to higher-level health care facilities. Antenatal care is essential for better health outcomes and reduces the incidence of maternal and perinatal morbidity. In 2018, a study was done regarding the association between antenatal care utilization (ACU) and maternal morbidity, which showed 34.6% women didn't receive adequate antenatal services so maternal morbidity was seen in 2.9% and neonates showed perinatal morbidity 5.5%.[13]

In this study, out of 262 females, 61 had their first antenatal visit to the hospital between 1 – 4 weeks. A similar study, conducted in Kenya showed only 14% of pregnant women opted for early antenatal care in the first three months of gestation.[14]

According to this research, 55.3% of pregnancies were planned. In Swaziland, a study was conducted among 1124 women and 70% of pregnancies were unplanned (teenagers and multiparas), and 30% were planned.[15]

Intake of iron and folic acid supplements is necessary for females but during pregnancy, body demands for these micronutrients increase significantly. In an Ethiopian study 2017, 28.7% of females of the reproductive age group took folic acid and iron.[16] In another cross-sectional study done in Uganda (2017) about 12% of the mothers attending antenatal clinic adhered to iron supplements.[17] Contrary to the results of the present study where 66% were taking folic acid and 69.8% were taking iron supplements. The major reason could be better awareness because it was an urban population and the majority were educated mothers.

Increased calcium intake is also a requirement of pregnancy. Calcium helps in fetal bone development. According to this study, 71.8% were taking calcium supplements during pregnancy. A study published in 2019 in Ethiopia, revealed that out of 492 pregnant females, 91% had calcium intake below the estimated average requirement.[18] The results of this study showed a significant association of education with the intake of oral supplementation during pregnancy.

According to this study, 58.8% of pregnant females vaccinated for tetanus and similar results were obtained in a study conducted in Egypt. It showed that out of 277 pregnant females 60.6% had taken all required doses of Tetanus Toxoid vaccine.[19]

The current study showed 21 % of pregnant females presented with hypertension as a complication of pregnancy whereas A study conducted in Greece showed 9.2% of pregnancies were complicated by hypertension.[24] Anemia is also aggravated during pregnancy. In our current study, 38.2% of pregnant women were found anemic. Similarly, in a previous study carried out in Faridabad, Haryana a high prevalence of anemia; 91.3% among pregnant ladies was noticed[25] and this observation is very common in developing countries. Contrary to the results of the present study, where only 2.29% of pregnant ladies had a history of sexually transmitted infections, a study in KwaZulu Natal, South Africa reported 32.3% of pregnant ladies were suffering from sexually transmitted diseases.[20]

Results of the current study showed a smaller proportion of women were affected with Tuberculosis and similar findings were observed from studies conducted in South India that showed only 0.02% of pregnant ladies were suffering from tuberculosis.[21] but this range is different in different parts of India. 6.1% of the pregnant ladies had a history of Hepatitis B infection. In this study whereas in Northwest Ethiopia 45.5% were diagnosed with hepatitis B.[22]

The results of this study showed that 12.9% had gestational diabetes and this incidence has been reported from 3.2% to 18% in different of the world.[23]

As satisfactory knowledge and awareness were noticed regarding institutional care, 96.6% of respondents were willing for hospital delivery. A study conducted in Kenya regarding the place of birth revealed out of 379 pregnant females, 103 (26%) delivered at home.[26]

LIMITATIONS OF STUDY:

1. This study is conducted in Lahore. Although the researcher has targeted Government-owned and private both types of hospitals to include a greater variety of participants whereas urban setting is a major limitation.
2. The hospital-based study does not reflect the true picture of responses so results can be affected by the selection technique.

CONCLUSION:

Education has a positive impact on good practices during the antenatal period.

RECOMMENDATIONS:

1. Enhancing the educational status of women can indirectly save maternal lives. Education has a great impact on safe practices during the antenatal period.
2. It is strongly recommended to create awareness through Public Education campaigns to increase knowledge of pregnant women at the household and community level for safe practices during the antenatal period.

AUTHOR'S CONTRIBUTION:

KA: Write up of introduction & extensive literature search
IM: Results analysis and write up of results, critical evaluation, finalization of article
AH: Conceptualization, supervision of project
QZ: Write up of discussion, literature search and referencing
MI: write up of methodology and data collection

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Orginal Article

ANTIOXIDANTS PREVENTED THE FETAL RESORPTIONS INDUCED BY SODIUM ARSENATE IN ALBINO MICE

Fariha Qureshi[1], Mohammad Tahir[2].

ABSTRACT:

Background and Objectives: Epidemiological studies have revealed the increased prevalence of spontaneous abortion, stillbirth, and premature babies among women who were exposed to high levels of arsenic in consumable water during their reproductive years. The study explored the fetal toxicity in albino mice inoculated by sodium arsenate and its prevention by Vitamins C & E.

Material and Methods: Gravid albino mice of BALB/c strain twenty-four in number were randomly distributed into 4 groups containing 6 animals in each group. Control group 1 was injected with distilled water 0.1ml/kg/day I/P for 18 days. A single dose of sodium arsenate 35mg/kg was injected I/P on 8th gestational day to groups 2, 3 & 4. Vitamins C and E 9 mg/kg/day and 15 mg/kg/day respectively, were given by intraperitoneal injections to groups 3 and 4 starting from 8th gestational day and continued for the rest of the pregnancy period. The fetal resorption sites were counted both early & late, litter sizes were logged. Morphological malformations were examined grossly.

Results: An increased incidence of abortion, fetal resorptions, and a significant decrease in litter size were manifested in group 2. Groups 3 & 4 showed noticeable improvement in litter size and the number of fetal resorptions were reduced. There was a statistically significant difference in means among the groups ($p<0.000$).

Conclusions: The results exposed the antioxidant potential of ascorbic acid and alpha-tocopherol in inhibiting the arsenic borne fetal toxicity in mice.

Key Words: Fetal resorptions, Antioxidants, Alpha-tocopherol

INTRODUCTION:

Arsenic is among the harmful substances in the environment, its inorganic salts are highly toxic and water-soluble.[1] These salts have the potential to cause structural or functional defects in conceptuses, abortion, and infertility in humans and animals.[2,3] In many countries of the world humans are susceptible to arsenic in clean water above the approved level (10µg/lit), which is associated with the development of skin and cancers of various organs.[4,5] The population of South East Asia, the West Bengal India and Bangladesh are more vulnerable to arsenic contamination in drinking water where its concentration at certain places rises to > 100 µg/lit.[6,7] In Pakistan the concentration of arsenic in water sources is found to be much higher (32-1900µg/l) than the permissible level in 27 districts along the course of river Indus and northern Pakistan.[8,9] In Pakistan 47 million people are vulnerable to arsenic through the contaminated groundwater wells which is above the WHO permissible level (10µg/l).[10]

Epidemiological studies carried out in Bangladesh, Nigeria, Romania, and Hungary had suggested associations between a high concentration of arsenic in consumable water and spontaneous abortion, still and premature births.[11-14]

Human and animal data from various studies supported the association between the detrimental reproductive effects and drinking water soiled with arsenic.[15] Anisur Rahman 2010, conducted a cohort study in Bangladesh and reported that prenatal arsenic exposure resulted in a decrease in size at birth.[16]

Hans ZJ 2011, demonstrated that prenatal arsenate exposure in chick embryos resulted

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in neural tube defects owing to arsenic-induced oxidative stress.[17] Robinson JF 2011 revealed the specific gene response in mouse embryos to different doses of arsenic and cadmium during the process of neurulation.[18] Various studies have documented the loss of human pregnancies with consumption of groundwater soiled with arsenic.[19]

In another study male Wistar rats were exposed to arsenic compounds in different concentrations in drinking water for 56 days, resulting in a decrease in reproductive functions and fertility.[20] A case-control study conducted in Egypt showed a positive correlation of fetal growth retardation and high concentrations of heavy metals including arsenic in blood and urine samples of 60 women.[21] Arsenic compounds in different concentrations were fed to rats for 6 weeks prenatally and during the gestation, fetal resorptions, abortions, decrease in fetal weight and cardiac malformations were reported.[22]

Arsenic induced these effects due to chromosomal damage and enhances mutagenesis by interfering with the DNA repair due to the production of free radicals.[23] Arsenate is a chemical analogue to phosphate; it disengages oxidative phosphorylation by replacing for phosphate in ATP synthesis.[24]

Antioxidants can prevent the damaging effects of free radicals by inhibiting oxidation reactions.[25] Heavy metals exert their toxic effects by generating free radicals which could be scavenged by antioxidants.[26]

Tsang V et al., 2012, evaluated the effects of gestational inorganic arsenic and high doses of folate on DNA methylation in mice. They reported adverse effects on DNA methylation.[27]

McDougal et al., 2017, established in zebrafish embryos that deficiency of vitamin E resulted in fetal resorptions, mortality & malformations.[28] Flora G et al., 2015, proposed therapeutic measures for chronic arsenic poisoning by the combination of different chelating agents.[29]

The chelating agents are itself teratogenic and couldn't be used effectively during pregnancy to prevent arsenic toxicity; therefore the research was aimed to investigate the antioxidant potential of Vitamins C & E in averting the damaging outcomes of free radicals induced by arsenic and subsequently prevent the fetal toxicity.

MATERIAL AND METHODS:

The albino mice of BALB/c strain (twenty-four females and eight males), were kept in the animal husbandry of the University of Health Sciences, Lahore under a controlled environment (temperature 22 ±1˚C and humidity 40%-60%) with a 12-hour light and dark cycle. The animals were 10 weeks old, weighed 30-35gm, were nurtured on customary pellet rodent diet and distilled water ad libitum. After the acclimation period of seven days, female mice were mated overnight with male mice of the same strain. Gestational day (GD) one was designated to the day when the copulatory plug was identified. The mice with positive copulatory plugs were randomly allocated into four groups with six faunas in each group. Cage cards were used to indicate the number of the mouse and its group. The control group 1was given weight-related distilled water by intraperitoneal injection, for 18 days. Mice of group 2 were injected with sodium arsenate 35 mg/kg by a single I/P injection on the 8th day of gestation; sodium arsenate was dissolved in distilled water before injecting. Animals of groups 3 and 4 received sodium arsenate 35 mg/kg on 8th GD by I/P injection and Vitamins C and E 9 mg/kg/day and 15 mg/kg/day respectively, from 8th day for the remainder of the gestation period. The dose was adjusted individually according to the weight of each dam.

The animals were dissected on the 18th day of conception. The uterine horns were exposed which appeared beaded by the fetuses. Uterine horns were incised in midline and examined for the number of live and dead fetuses. Gross morphological examination for malformations of all fetuses

was carried out under a Wolfe stereo dissecting microscope, ER- 59 – 1828, and the following parameters were looked for: i. Exencephaly ii. Cleft palate. iii. Abdominal hernia. iv. Polydactyl & Opened eyes. The total numbers of litters in each group were recorded and their means were calculated. The uterine horns were also examined for the early and late fetal resorptions, the number of fetal resorptions/dams were documented, and a mean of the total number of fetal resorptions was calculated. The uterine horns which lacked the mark of implantation were exposed and were put in 10% ammonium sulfide solution for revealing early implantation sites.

The software (SPSS) version 18.0 was made use of to analyze the data. For the numerical variables mean and standard deviations were calculated. ANOVA was used to evaluate the mean difference among the groups. Post-hoc Tukey was tested to assess the difference of means between the groups. The p value of ≤ 0.05 was contemplated as statistically significant.

RESULTS:

In the control group 1 there was no occurrence of abortions, stillbirths, fetal resorptions, or maternal mortality. The uterine horns were opened up on day 18 of pregnancy which exhibited the normally growing fetuses (Fig 1, A).

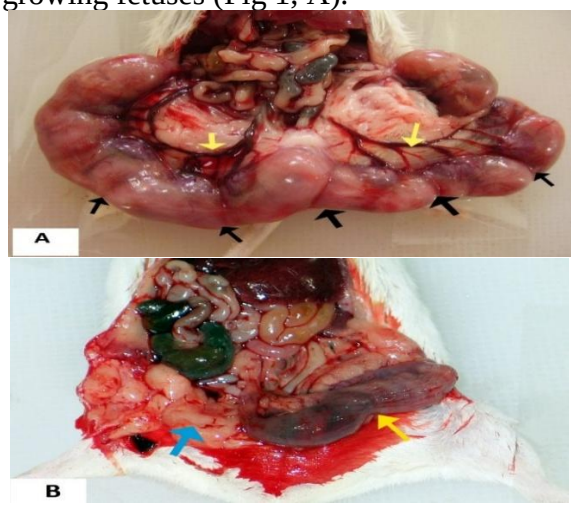

Fig 1: Photographs of mice on day 18 of pregnancy. A) Shows the dissected mouse of control group 1, displaying the uterine horns blood circulation through the uterine blood vessels (yellow arrows) on the mesometrial side of the uterus. Fetuses have seen growing normally through the wall of the uterus giving it a beaded appearance (black arrows). B) Dissected mouse treated with sodium arsenate (group 2), the right horn of uterus displays bleeding from the aborted fetuses (blue arrow). The left uterine horn shows a few of the remaining fetuses (yellow arrow).

In sodium arsenate treated group 2 there were spontaneous abortions, therefore more animals were added to the group (n=10) to balance the number of the group. The animals started aborting on 17th and 18th gestational days. The mice were dissected which showed bleeding from the aborted fetuses and a few numbers of partially formed fetuses (Fig1, B). In groups 3 and 4, the sodium arsenate was administered in consort with Vitamins C and E respectively; no incidence of spontaneous abortions or maternal mortality was observed in these groups. The fetuses were well developed with all normal body parts. There was no incidence of stillbirth nor was there any evidence of exencephaly, cleft palate, abdominal hernia, polydactyl, or opened eyes (Fig 2, A&B).

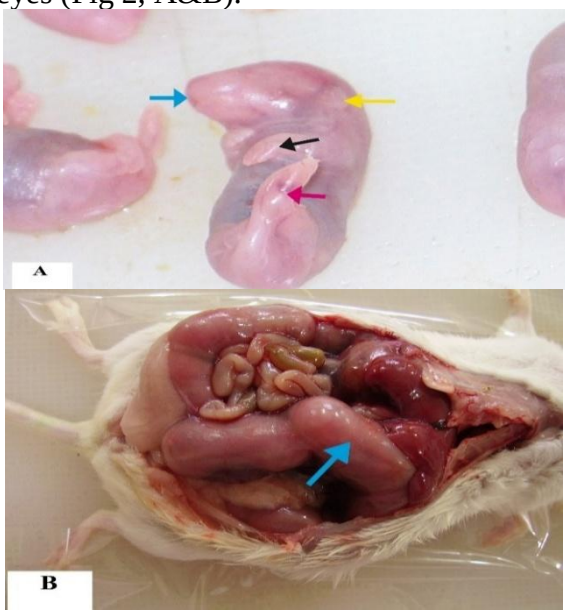

Fig 2. Photograph of mice fetuses from groups 3&4. A) Shows a fetus; with well-formed jaws (blue arrow), ear (yellow arrow) forelimbs (black arrow), turned up tail (red arrow). B) A dissected female

mouse, showing well-formed fetuses in uterine horns (blue arrow) occupying the lower abdomen.

In sodium arsenate treated group 2 early and late fetal resorptions were observed. The mean number of resorptions among various groups was statistically significant (Table 1, Fig 3 A& B).

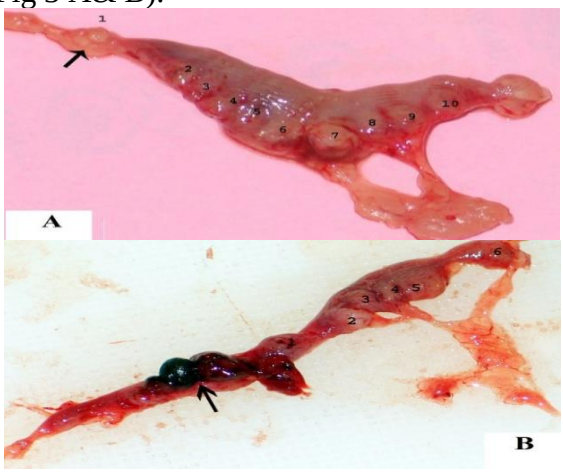

Fig. 3. Photograph of uterine horns of mice (Group 2). A) Showing resorptions at the implantation sites; the opened up uterine cavity showing site of early resorption turned into the yellow fat body (arrow), and the number indicating the sites of resorption. B) Uterine horn, dissected to show the late resorptions (arrow) and the number indicating sites of early resorptions.

Table 1: Comparison of fetal parameters among various groups.

Parameters	Control group 1 (n=6)	Sodium arsenate group 2 (n=10)	Sodium arsenate + Vit C group 3 (n=6)	Sodium arsenate + Vit E group 4 (n=6)	p-value
	Mean ± SD	Mean ± SD	Mean ± SD	Mean ± SD	
Number of fetal resorptions.	0.00 ± 0.00	3.7 ± 2.6	0.33± 0.5	0.5± 0.8	p<0.000 *
Total number of fetuses.	9.5± 1.4	5.5± 1.8	10.2± 2.3	8.5± 2.2	p<0.000 *

The sight of resorption was made discernable by opening the uterine horns and placing it in a 10% ammonium sulphide solution. Groups 3&4 showed a minor number of resorptions. Post-hoc Tukey test applied for multiple comparisons among the groups showed a significant difference in mean of the total number of resorptions between the groups 1&2, 2&3, 2&4; the number of fetal resorptions was considerably higher in group 2where as it was reduced in groups 3&4; the difference of means of the total number of resorptions between groups 1& 3&4 was statistically insignificant (Table 2A).

Table 2A: Multiple comparisons of mean of the total number of resorptions among various groups according to the Tukey test.

Comparison among groups		**Mean Difference**	**Level of Significance**
Groups (α)	Group compared (β)	(α-β)	p-value
(1)	(2)	-3.7	0.001*
	(3)	-0.33	0.985
	(4)	-0.50	0.952
(2)	(3)	3.37	0.003*
	(4)	3.20	0.005*
(3)	(4)	-0.167	0.998

*The mean difference is statistically significant between groups 1&2, 2&3, 2&4. The mean difference is statistically insignificant between groups 1&3, 1&4, 3&4.

The average number of litters was reduced in sodium arsenate treated group 2 as compared to groups 1, 3&4. The comparison of means of the total number of fetuses among the groups was statistically significant (Table 1). The number of fetuses/dams was also more in groups 1, 3, and 4 as compared to group 2. The Post-hoc Tukey test was applied for multiple comparisons among the groups; there was a significant difference in mean of the total number of fetuses between the groups 1&2, 2&3, 2&4. (Table 2B). The data are given in (Fig 4).

Table 2B: Tukey test showing multiple comparisons of mean of the total number of fetuses among various groups.

Comparison among groups		Mean Difference	Level of Significance
Groups (α)	**Group compared (β)**	**(α-β)**	**p-value**
(1)	(2)	4.0	0.003*
	(3)	-o.7	0.930
	(4)	1.0	0.803
(2)	(3)	-4.7	0.000*
	(4)	-3.0	0.028*
(3)	(4)	1.7	0.450

* The mean difference is statistically significant between groups 1&2, 2&3, 2&4. The mean difference is statistically insignificant between groups 1&3, 1&4, 3&4.

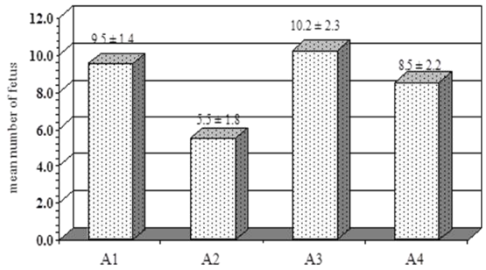

Fig 4. Bar chart showing the comparison of mean of the number of fetuses among various groups.

DISCUSSION:

In this study administration of intraperitoneal injection of sodium arsenate on 8th GD in group 2 resulted in a decreased number of litters, frequent fetal resorption, and spontaneous abortion. Spontaneous abortions and decreased fecundity in mice after arsenite toxicity which leads to the placental insufficiency attributing these effects have been reported.[19] The fetotoxic effects of sodium arsenate manifested as an increased rate of fetal resorption had been documented by Sampayo et al., 2017, Gandhi 2012 and Markowski 2011.[30-32] In our work the external malformations like craniofacial, skeletal, limb defects, abdominal hernia, polydactyly, and opened eyes were not observed as had been reported by Wlodarczyk 2014 & Javanmard 2011 .[33,34] This may be due to the high rate of resorptions and abortion. The sodium arsenate induced oxidative stress which causes DNA damage through the production of free radicals having possibly an effect on the developing embryo eliminating the abnormal conceptuses. Han Z et.al, 2011, revealed that the embryos with neural tube defects showed a significantly higher concentration of free radicals.[17]

In groups (3&4) sodium arsenate along with Vitamins C and E were injected respectively, there was a considerable increase in the mean of the number of litters as equated to group 2 and even greater than in the control group 1. There was no incidence of spontaneous abortion and the number of fetal resorptions decreased in groups 3&4. This suggested that due to their antioxidant properties the Vitamins C and E had prevented the fetotoxicity, avert the high level of free radicals in the body and block the DNA damage.[35] Davis 2010, discussed the significance of antioxidants to combat the hypoxic intrauterine environment and reported that surge of antioxidants like Vitamins C, E and A were found in the cord blood of term infants as compared to preterm infants.[36]

A fundamental balance between oxidant and antioxidant molecules is essential for a normal pregnancy to take place, interruption in this balance could contribute to defective embryo development.[37] In this study arsenic presumably broke down the balance between oxidant and antioxidant levels by producing free radicals and resulted in abortions and fetal resorptions, while the antioxidant potential of Vitamins C&E had inhibited this inequity of oxidant and antioxidant molecules and prevented the fetotoxicity.

CONCLUSION:

This study concludes that the spontaneous abortions & fetal resorptions induced due to free radical formation by arsenic, have been prevented by Vitamins C & E proving that the free radicals can be scavenged by these antioxidants & hence prevented the fetotoxicity. However, further studies to assess the effects of these vitamins are

requisite on human conceptuses in areas where women in their childbearing years are susceptible to arsenic through the polluted water supply.

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AUTHOR'S CONTRIBUTION:

FQ: Conduction of study, design, hypothesis formation, analysis & interpretation of data, drafting, revising critically & final submission of manuscript & responsible for correspondence as contributing author.
MT: Supervisor of the project. Integrity & technical aspects had been investigated by him.

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Original Article

COMPARISON OF ACADEMIC PERFORMANCE OF 1ST YEAR MBBS MALE AND FEMALE STUDENTS IN THE SUBJECT OF PHYSIOLOGY

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ABSTRACT:

Objective: To compare the academic performance of males with females in assessment tests of 1st-year MBBS in the subject of Physiology.

Subjects and Methods: It was a retrospective study conducted on the results of first-year MBBS students in the monthly assessment tests. After approval from the institutional research board, the results of monthly tests in five sessions 2013-14, 2014-15, 2015-16, 2016-17, and 2017-18 were included. Data were analyzed for the performance of girls and boys as a whole. Out of a total of 781 students admitted during these 5 years, 467 were females (60%) and 314 (40%) were males (fig. I). The performance of girls and boys was further categorized into four groups. G I: high achievers (marks ≥ 80%), G II: good students (marks70-79%), G III: average (50-70%), and G IV: poor (< 50%). The performance of girls and boys was assessed in each group and calculated as % age. The difference was tested by students' "t" test and a p-value of < 0.05 was regarded as significant.

Results: Among girls, the results were distributed into these 4 categories as follows: Group I= 0, Group II= 45 (9.6%), Group-III= 353 (75.6%) and Group IV = 69 (14.8%). As for the boys, the performance of each group was, Group I= 0, Group II = 7 (2.22%), Group III = 221 (70.39%), and Group IV = 86 (27.39%) boys respectively. (Table 1, fig 1) T-test value on 2 sample data was 7.5440, p-value = 0.00 (highly significant).

It was also observed that the first and second test showed an overall good result from both genders. The third test which was conducted after sports week in all the five sessions showed a decline in the performance of both the genders. The tests held after spring and summer vacations also had comparatively lower scores. The girls maintained their slight supremacy in these results as well. (Fig 3)

Conclusion: The academic performance of girls is significantly better than that of boys in all groups (p-value = 0.00). Both the groups give low performance after social events in college but finally cover up their deficiencies.

Key Words: Gender, Academic Performance, Medical Education

INTRODUCTION:

Education plays an important role in the lives of individuals to make them useful members of society.[1] It imparts knowledge, skills of reasoning, values, self-control and capacity of healthy social interaction and is processed at the level of the home, school and community.[2-3]

The academic performance of a student depends on multiple factors. These include the environment of an institution, its facilities, and discipline.[1] Students' desire to demonstrate their competency to teachers and parents, self-satisfaction, and the amount of hard work and dedication they put in also matters.[4] Females have been observed to be more dedicated to their education and work hard than males.[5] The number of females getting admission in

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medical education based on competency is increasing worldwide.[6-8] In a developing country like Pakistan, gender discrimination has caused hurdles in female education in the past.[9] The parents are paying the fees of private medical colleges to educate their children and this accomplishment matters a lot to them as well as the institution. This study was conducted to assess any difference in performance between the two genders in the last five sessions. It may help to take measures to improve the standard of those who are weak.[9]

MATERIAL AND METHODS:

This retrospective study was conducted at Akhtar Saeed Medical and Dental College in 2019. Approval from the institutional research board was taken. The sample was picked up using a universal sampling technique. Out of a total of 781 students, there were 467females (Girls) (60 %) and 314 males (Boys) (40 %) (Fig 1). The data comprised of results of all physiology tests of first-year students from the sessions 2013-14, 2014-15, 2015-16, 2016-17, and 2017-18. The results of monthly class tests for each student were summed up and an average % age was calculated. It was then divided into four groups. Data were categorized into four groups. Group-I included the number of students securing more than or equal to 80% marks, Group-II between 70 to 79%, Group-III between 50 to 69%, and Group IV 50% marks.

RESULTS:

Among Females, the results were distributed into these categories as follows: Group I = 0. Group II = 45 (9.6%), Group III = 353 (75.6%) and Group IV = 69 (14.8%). As for the male students the performance in each group was, Group I = 0, Group II = 7 (2.22%), Group III = 221 (70.39%) and in Group IV = 86 (27.39%) boys respectively. (Table 1, fig II) student's t-test value on 2 sample data was 7.5440, p-value = 0.00 (highly significant).

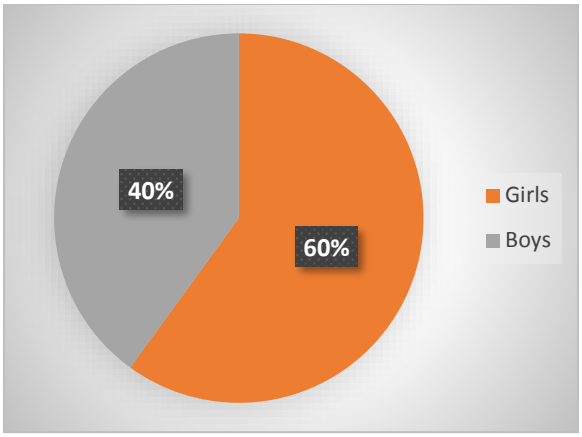

Fig 1. Percentage of Admission of the two genders in five years.

Table 1. Comparison of cumulative academic performance of Girls and Boys in monthly tests.

Groups	Girls (n=467)	Boys (n=314)
I (≥80%)	0	0
II (70-79%)	45	7
III (50-69%)	353	221
IV (<50%)	69	86

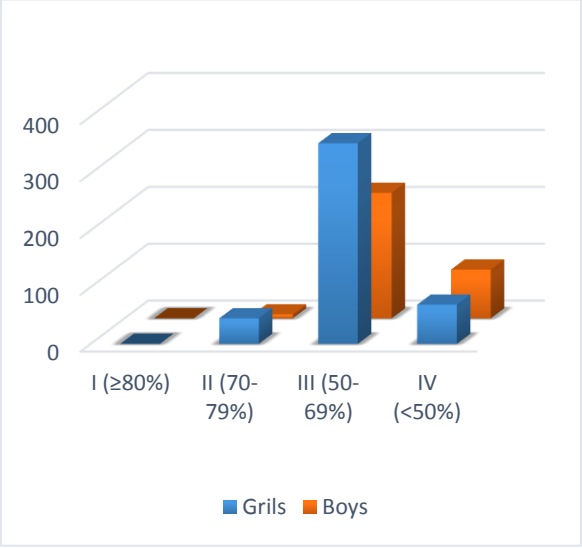

Fig 2. Comparison of academic performance of Girls and Boys (percentages) in monthly tests.

Table – 2. Overall pass percentage of Girls and Boys in Monthly Tests of five years.

Monthly Tests in five years	Girls Pass %	Boys Pass %	p-value
1	89.50749465	83.75796178	0.004**
2	89.50749465	78.66242038	0.00001**
3	80.0856531	65.2866242	0.000008**
4	84.18230563	70.98039216	0.0002**
5	71.52034261	69.74522293	0.13*

*Non significant **Significant

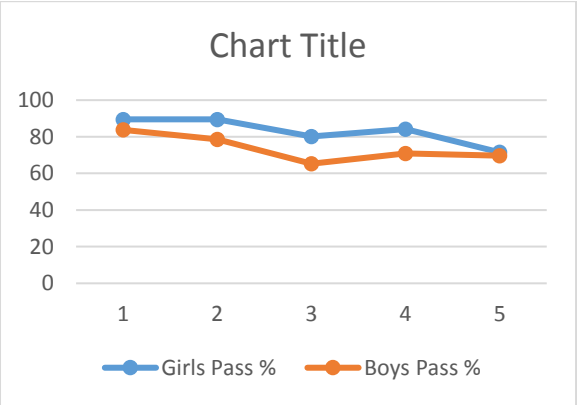

Fig 3. Overall pass percentage of Females and Males in Monthly Tests of five years.

It was also observed that the first and second test showed an overall good result from both genders. The third test which was conducted after sports week in all the five sessions showed a decline in the performance of both the genders. The tests held after spring and summer vacations also had comparatively lower scores. The females maintained their significant supremacy in these results as well. (Table 2, Fig 3)

DISCUSSION:

Education is a process devised by man to improve upon its standards in respect of earning and social life.[9] The medical education helps in getting respectable jobs along with a chance to serve humanity.[10-11] Success in the field of medicine requires highly competent and hardworking individuals. Most of the educational institutions teaching medicine are concerned about the factors that could influence their results.[6] There have been many studies that compare the performance of the two genders in the field of social sciences, mathematics, medicine, and others and gave variable results.[12-14] Worthy of note is the fact that an increasing number of female students are opting for this field and succeed in getting admission on basis of merit.[6-8] Our study included a total of 781 students who were enrolled in this institute during the 5 years (2013 to 2017). There were 467 (60%) females and 314 (40%) males showing a rise in the number of female students in accordance with the previous studies.[6-8] The female students showed a significantly better result (p-value = 0.00) than males throughout the academic year as confirmed by similar findings in some other studies.[5-8,13] The results of one study showed no such difference. We can attribute their result to a different set of social values in the community of KPK where females have just started to surge up from an era of suppression. There was a significant decline in the performance of both genders after social activity week but then attained the previous level afterward.

CONCLUSION:

The academic performance of girls was significantly better than that of boys in all groups (p-value = 0.00). Both the groups gave low performance after social events in college but finally covered up their deficiencies.

AUTHOR'S CONTRIBUTION:

SM: Planing and collection of data, drafting of article
MK: Checking plagiarism and frazing of article
AF: Data collection and bibliography
MQ: Statistical Analysis and reviewing of article
MT: Data collection

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Original Article

EFFECT OF ETHANOLIC EXTRACT OF CLOVE (Eugenia Caryophyllata) ON PAIN IN MICE

Saima Tabassum[1], Hamid Javaid Qureshi[2], Ambreen Anjum[3], Sobia Manzoor[4], Hafiza Hina Pasha[5], Wardah Toseef[6]

ABSTRACT:

Background: Pain is a natural phenomenon. There are several pharmacological medicines available in the market for pain relief. Ethanolic extract of clove is a natural ingredient and can be as beneficial as a pharmacological drug for pain relief.

Objective: To determine the effect of Eugenia caryophyllata flower buds ethanolic extract on experimentally induced pain in albino mice.

Material and Methods: This randomized trial was done on 90 male albino mice. An intraperitoneal injection of 0.6% acetic acid was induced for the writhing test as a chemical model of nociception. Mice were divided randomly into three equal groups. Group A was considered as a control group (n=30) and normal saline was infused; group B was given Eugenia caryophyllata flower bud ethanolic extract (n=30) and group C was given an intraperitoneal injection of indomethacin (n=30). In these mice, abdominal contractions (writhings) were counted. SPSS version. 22 was used to analyze the data.

Result: The mean number of writhing in each of the three groups of mice was 16.80 writhings in group A, 4.90 writhings in group B, and 4.60 writhings in group C. Ethanolic extract of clove and indomethacin significantly reduced ($p<0.05$) the number of writhing.

Conclusion: The Eugenia caryophyllata ethanolic extract significantly reduces the pain in mice. This analgesic effect is almost similar to that produced by indomethacin.

Key Words: Clove, Pain, Mice

INTRODUCTION:

Pain is a natural emotional and sensory experience, related to the actual or potential tissue injury. It is an essential feature of defense mechanisms of the body to lessen the physical harm.[1]

Analgesics or opioids are the most common therapeutics which can help to relieve the pain. Numerous researches have already been done on these to relieve pain.[2]

The bioactive composites present in edible as well as medicinal herbs are valuable molecules for the synthesis of many medicines containing activity against many syndromes, especially involved in inflammation that is associated with oxidative stress.

Numerous such herbs have a considerable inhibitory effect on inflammatory response and oxidative stress and can help protectively to increase the quality of life after initiation of taking diets, which is rich in such components.[3]

There are several natural herbs, which have analgesic properties and are traditionally used without any sort of adverse effects. Clove is the most valuable spices which have been used for several centuries, especially in tropical countries for food preservation and medicinal purposes.[4]

Eugenia caryophyllata (Clove), is a medicinal plant, which is traditionally used to avert the pain. It is commonly found in

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tropical countries. Conventionally, flower buds of the clove are used in medicine for the management of rheumatic pains, sciatica, headache, neuralgia, toothache, indigestion, nausea, loss of appetite, hiccup, vomiting, paralysis, and skin disorders.[5]

Clove is used as a medicinal herb, from several years in Chinese traditions. Cloves have antiseptic, antifungal, antibacterial and anti-viral action.[6,7] Chemical composition of clove bud is diverse. It is composed of carbohydrates, fat, proteins, and water. It also has minerals including sodium, calcium, potassium, iron and phosphorous it also contains some vitamins like riboflavin, thiamine, ascorbic acid, niacin, and vitamin A.[8]

Several properties of clove bud including antiseptic, antifungal, antiviral, antibacterial, antipyretic, anti-oxidant, anti-allergic, anticonvulsant, anti-mutagenic, insecticidal and natural anti-helminthic have been studied extensively in the world,[9,10] but to our knowledge, in Pakistan, no research has been done on the above-stated properties of clove. The effects of Eugenia caryophyllata extracted oil and Ethanolic extract on pain and inflammation are encouraging.[11]

In our country, the abuse of analgesic drugs has increased in previous years. This may be because of the over-the-counter sale and purchase of these medicines. These drugs are causing an increase in gastro-intestinal tract problems including gastritis, gastric ulcers, bleeding, and renal damage, and failure of some other organs. Medicinal herbs can be a good replacement for pharmacologically based analgesics and are also safe and cost-effective.

MATERIAL AND METHODS:

This Randomized controlled trial was done at the Department of Physiology, Services Institute of Medical Sciences, Lahore. The trial was done on 90 adult male albino mice, Mice were distributed in three equal groups of 30 mice in each group mice were kept in a cage for a week beforehand initiation of the trial. Atmospheric conditions were maintained at the 24 ± 2^0C and dark: 10-12 hours darkness and 12-14 hours light. All mice were given standard pellet diet ad libitum which was commercially available and tap water in clean bottles was given.

Eugenia caryophyllata flower buds (3000g in dried condition) were obtained from the local market. The extract of Eugenia caryophyllata flower buds was made using ethanol and standardized by using the facilities available at Applied Chemistry Research Centre, PCSIR Labs, Lahore.

The mice were divided randomly into three groups. Each contained 30 mice:

Group A (Control): given intra-peritoneal normal saline, 10ml/Kg

Group B (Experimental): given intra-peritoneal ethanolic extract of Eugenia caryophyllata flower buds, 50mg/Kg

Group C (Reference): given intra-peritoneal indomethacin, 3mg/Kg

After the administration of the trial drug, each mouse was transferred to a separate, transparent glass case and trained for 30 minutes. Then, acetic acid (0.6%) in a dose of 10ml/Kg was injected intraperitoneally and writhings (abdominal contractions) were counted for 15 minutes. Percentage inhibition of writhing was calculated to observe the analgesic effect by using the following formula:

$$\text{Inhibition } (\%) = (1 - Wt/Wc) \times 100$$

Where Wt and Wc represent the number of writhings in experimental (Eugenia extract or indomethacin) and control group, respectively. SPSS v. 22 was used to analyze the data and the mean number of writhing was compared by using one-way ANOVA tests.

RESULTS:

The mean number of writhing per 15 minutes was 16.80±0.21 in the control group, 4.90±0.14 in the Eugenia extract group, and 4.60±0.18 in the indomethacin group. The percent inhibition in the Eugenia group was 70.83% while 72.62% in the indomethacin group ($p=0.000$), indicating a highly significant analgesic effect in these groups. (Table 1)

Table 1. Comparison of three groups for body weight, number of writhing, and percentage inhibition of writhing among mice.

Param-eter	A (Cont-rol)	B (Eugenia (Caryo-phyllata)	C (Indometha-cin)
	n=30	n=30	n=30
Number of writhing (in 15 min)	16.80± 0.21	4.90±0.14*	4.60±0.18*
Percent Inhibition (%)	0.00	70.83	72.62

*p=0.000, highly significant in comparison to control

DISCUSSION:

Pain is usually defined as ache prolonged for ≥3 months.[12] Opioids are used for effective control of pain, but the evidence available in literature does not recommend the prolonged use of such opioids for the treatment of chronic non-cancerous pain. The patients taking opioids for a prolonged period have a high risk of opioid use disorders also some other adverse outcomes.[13-15] Side effect such as addiction should be avoided.[16]

For medical use, several herbs can be taken orally, sublingually, or even topically and can also be smoked; inhaled; combined, or cooked with food or drinks. These can be used in herbal form, extracted naturally from the plant, or prepared synthetically.[2]

Eugenol (4-allyl-2-methoxyphenol) is the phenolic compound from phenylpropanoids class and the main component of the clove.[17] Eugenol is used in the food industry as a preservative, primarily because of antioxidant properties, for flavoring of foods, and also in cosmetics.[18] Furthermore, clove is also known for its anti-inflammatory activities, which might be due to anti-inflammatory actions of eugenol.[19]

In the present study, intraperitoneal administration of the ethanolic extract of clove flower buds significantly decreased the number of writhing (abdominal contractions) as compared to the control with the percentage inhibition being 70.83%. This inhibition (i.e. 70.83%) is more than that reported by Daniel (61.6%) who used essential oil of Eugenia caryophyllata but this inhibition is less than that reported by Tanko et al. (i.e. 75%) who used the ethanolic extract of clove flower buds and observed maximum effect by the same dose which has been used in this study (i.e. 50 mg/Kg).[20] The percentage inhibition of writhing caused by the reference drug indomethacin was 72.62%.

Taher et al. found that in the mice given Eugenia caryophyllata extracted oil, acetic-acid-induced writhing was reduced significantly by 87.7% ($p<0.01$) than 77.7% produced ($p<0.01$) by 100 mg/kg, intraperitoneal aspirin injection. Additionally, Eugenia caryophyllata oil, as well as indomethacin, have anti-inflammatory effects, i.e. 50.6% ($p<0.05$) and 70.4% ($p<0.01$), respectively, to avert the edema of mouse foot, which was induced by using carrageenan.[21]

Clove extract is enriched with polyphenol, because of its antioxidant property, the clove extract is capable to inhibit the secretion of advanced glycation end products and protein glycation. Such findings recommend the use of clove extract for some diabetic complications.[5]

Daniel et al in 2009, used essential oil of Eugenia caryophyllata flower buds. In an acetic acid-induced writhing test to determine the analgesic effects, 61.6% inhibition was achieved.[22]

Eugenol is extensively used in dentistry as a local analgesic agent, owing to its ability to lessen the toothache. Interestingly, eugenol shares many pharmacological activities with local anesthetics. It inhibits voltage-gated sodium channels and sensory receptors

which are involved in the perception of pain, the transmission of action potential.[23]
Ethanolic extract of the Eugenia caryophyllata flower buds possesses a potent analgesic effect and it can be used as a traditional remedy for different pain disorders.

CONCLUSION:

The ethanolic extract of Eugenia caryophyllata significantly reduces pain in mice. This analgesic effect is almost similar to the effect of indomethacin.

ACKNOWLEDGMENT:
We pay special thanks to Dr. Zaheer-ud-Din Khan, Professor & HOD, Department of Botany, Government College University, Lahore for identifying the type of flower buds used in the experiment. We are also thankful to Applied Chemistry Research Centre, PCSIR Labs, Lahore for extracting the ethanolic extract of clove**.**

AUTHOR'S CONTRIBUTION:

ST: Conception of idea and study deisgn
HJQ: Supervisor of the reserach work
AA: Data collection
SM: Data analysis
HHP: Data collection
WT: Drafting the arcticle

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Original Article

ROLE OF PROGNOSTIC VARIABLES OF MEDICAL IMPORTANCE AND THEIR INTERPLAY IN LEUKEMIA: A STUDY FROM LOCAL POPULATION

Rabail Alam[1], Muhammad Saeed Qureshi[2], Zunaira Kanwal[3], Sulayman Waquar[4], Saima Iqbal[5], Naeem Farooq[6]

ABSTRACT:

Introduction: Leukemia is defined as the cancer of blood-forming tissues. It is equally common in children and adults. It involves abnormal production of white blood cells (WBCs) which are primarily responsible for the defense in the human body thus, abnormality in the production of WBCs leads to the failure in combating the infection. Aim of the current study is to rule out the significant markers of prognostic importance that play an important role in the development of leukemia in the local population

Material and Methods: Thirty (n=30) patients of leukemia and thirty (n=30) healthy controls were enrolled for the current study by random sampling. This cross sectional study was approved by the Departmental Research Committee (DRC), Institute of Molecular Biology and Biotechnology (IMBB), the University of Lahore. Blood and Saliva samples were collected and subjected for the analysis of the MDA, isoprostanes, Interleukin, MPO, and Neutrophils levels with the help of their respective protocols.

Results: Results of this study showed that the levels of oxidative stress markers and interleukins were significantly increased in patients with leukemic disorders as compared with the healthy subjects. It showed that levels of MDA, isoprostanes, 8-OHdG, TNF-α and interleukin-6 were significantly higher (p-value = 0.019, 0.001, 0.041, 0.008 and 0.016 respectively) in the serum and saliva samples of patients as compared to that in the healthy subjects. Levels of MPO and Neutrophils presented significantly (p-value= 0.043, 0.007) higher levels in the blood samples whereas, these were not detected in the saliva samples of the patients.

Conclusion: The current study suggests the significant role of oxidative stress markers in the initiation and progression of leukemia. It shows levels of interleukin and markers of DNA damage remained elevated in the patients with leukemia as compared to that of healthy individuals. Therefore, therapy with significant antioxidants can improve the status of individuals suffering from leukemia in the local population.

Key Words: Leukemia, Neutrophils, Interleukin-6

INTRODUCTION:

Leukemia is a Greek word meaning 'leukos=white' + 'haima=blood'. As the name indicates leukemia refers to the cancer of bone marrow i.e. leading to a wild proliferation of blood-forming cells. Bone marrow cells include white blood cells (WBCs) which combat infection, red blood cells (RBCs) which carry oxygenated blood and platelets which aid blood clotting.[1]

Major types of leukemia are acute myelogenous leukemia (AML), chronic myelogenous leukemia (CML), acute lymphoblastic leukemia (ALL), and chronic lymphoblastic leukemia (CLL). In all its types bone marrow problem leads to excessive blood cells in the bloodstream by favoring leukemic stem cells and bone marrow fibrosis. The most common type of

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leukemia diagnosed is acute lymphocytic leukemia, which includes 78% of all detected children leukemias.[2] The prevalence of acute lymphocytic leukemia in elder patients in every 100,000 patients is 1.0 to 1.6 which is higher as compared to patients aged 25-54 (0.6 to 0.7) as reported by surveillance epidemiology and end-result study.[3] While acute myeloblastic leukemia (AML) is about 20% of pediatric leukemia.[4] Reactive oxygen species (ROS) are diverse compounds produced by the mature myeloid cell lines in an innate response. They play a role in the signaling process either intracellular or extracellular, exogenously or endogenously.[5] Oxidative stress due to ROS is responsible for DNA damage.[6] Oxidative stress may be held accountable for defective signaling mechanisms that alter the efficacy of drugs and programmed cell death of malignant cells.[7] Thus, antioxidants play their pivotal role in altering the anomalies that may be caused by the production of reactive oxygen species i.e., elevated levels of Superoxide dismutase (SOD), Glutathione (GSH) and Catalase (CAT) have reported grasping effect on the oxidative stress, in case of lower levels of these anti-oxidants enhanced progress of diseases pathogenesis and aging are reported.[8] Extensive literature signifies the interactions with the bone marrow microenvironment that is responsible for the hematopoiesis and morphology of bone marrow. Elevated levels of transforming growth factor beta-1 are important to control cell proliferation, survival and apoptosis.[9] Literature reports the role of various markers such as interleukins, isoprostanes (Iso-P), 8-hydroxy-2-deoxyguanosine (8-OHdG), Tumor Growth Factor-beta (TGF-β) have a significant role in the disease progression.[10] Markers like 8-OHdG and Iso-P signifies increased lipid peroxidation and DNA damage in the cells of the infectious patients. As reported by the number of studies lipid peroxidation by-products i.e., MDA is involved in the formation of DNA adducts leading to DNA damage and cell death.[11]

MATERIAL AND METHODS:

Thirty (n=30) patients of Leukemia and thirty (n=30) healthy age-sex matched controls were enrolled in the current study. After getting informed consent blood and saliva samples were obtained and stored for their future analysis. All of the protocols were approved by the Departmental Research Committee (DRC) of the Institute of Molecular Biology and Biotechnology (IMBB), The University of Lahore. Samples were subjected to the determination of Malondialdehyde (MDA), isoprostanes (IsoP-F2α), 8-hydroxy-2-deoxyguanosine (8-OHdG), Interleukin-6 (IL-6), Tumor Necrosis Factor-alpha (TNF-α), Myeloperoxidase (MPO) and Neutrophils with the help of their respective ELISA and spectrophotometric methods. Results of the findings were subjected to Independent T-test with the help of SPSS v.21 and were expressed in the form of Mean±S.D. where $p<0.05$ remained significant.

RESULTS:

TABLE- 01: Levels of different variables in leukemia

Variables	Control (n=30)	Serum (n=30)	Saliva (n=30)	p- value
MDA (nmol/ml)	0.95± 0.001	5.26± 1.26	1.26± 0.05	0.019
IsoP- F2α (ng/ml)	0.99± 0.0056	81.26± 5.26	4.26± 1.49	0.001
8-OHdG (pg/ml)	0.02± 0.0011	1.22± 0.016	0.06± 0.001	0.041
IL-6 (pg/ml)	4.26± 1.06	6.59± 2.16	0.965± 0.16	0.016
TNF-α (pg/ml)	26.25± 3.26	56.26± 2.26	0.15± 0.015	0.008
MPO (mmol/L)	1.56± 0.052	2.16± 0.16	0.00± 0.00	0.043
Neutrophils (%)	60.31± 3.06	88.16± 3.26	0.00± 0.00	0.007

The current study showed that serum MDA levels were increased significantly (p=0.019) in patient as compared to controls. Whereas an insignificant increase was observed in saliva of patients (Fig. 1)
Serum Isoprostanes was significantly higher (p=0.001) in patient as compared to controls. In saliva, its level was slightly increased. (Fig. 2)
Levels of serum 8-OHdG were signifincatly higher (p=0.041) in patients as compared to values of controls. While it was slightly detectable in saliva. (Fig. 3)
Levels of IL-6 were not detectable in saliva but were significantly higher (p=0.016) in the serum of patients as compared to controls. (Fig. 4)
Levels of serum TNF-α were significantly elevated (p=0.008) in patients as compared to controls. There was no effect on slivery TNF-α. (Fig. 5)
Serum MPO levels were significantly higher (p=0.043) as compared to controls. There was no effect on salivary MPO. (Fig. 6)
Neutrophils percent was significantly higher (p=0.007) as compared to controls. No neutrophil was detected in saliva. (Fig. 7)

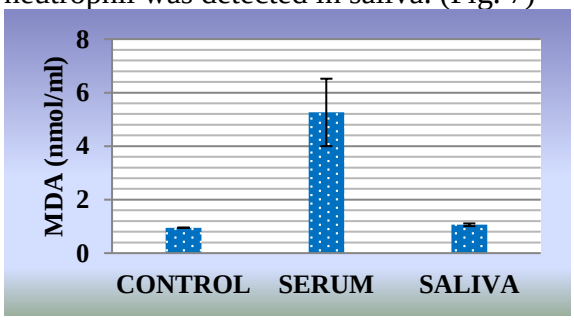

Fig. 1 MDA levels in serum and saliva of patients.

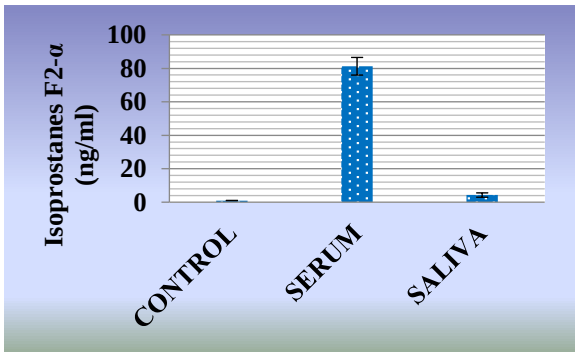

Fig. 2. Isoprostanes levels in serum and saliva.

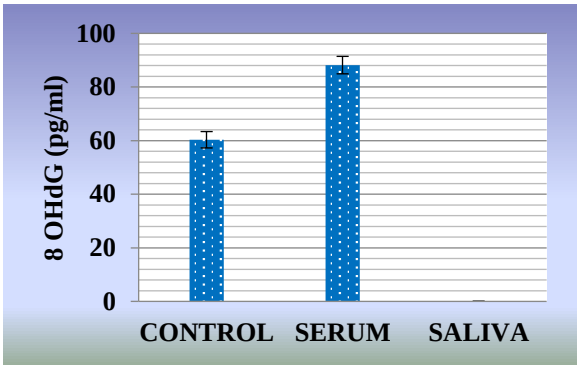

Fig. 3. 8OHdG levels in serum and saliva.

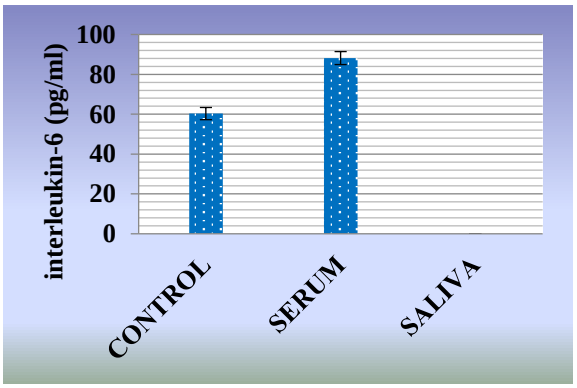

Fig. 4. Serum interleukin-6 levels in serum and saliva.

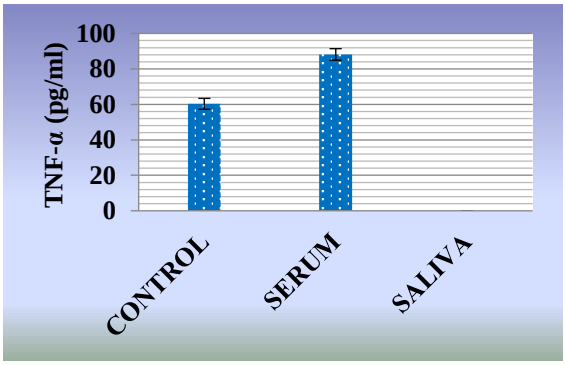

Fig. 5. Serum TNF-α levels.

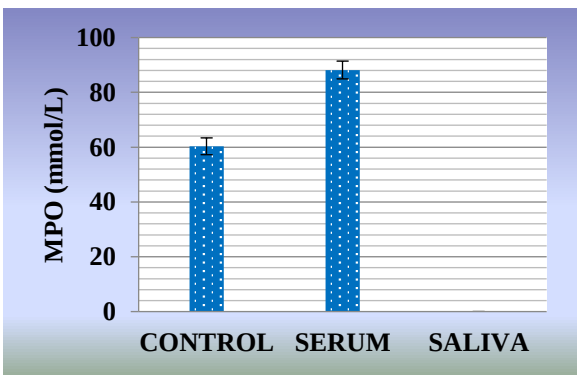

Fig. 6. MPO levels in serum and saliva.

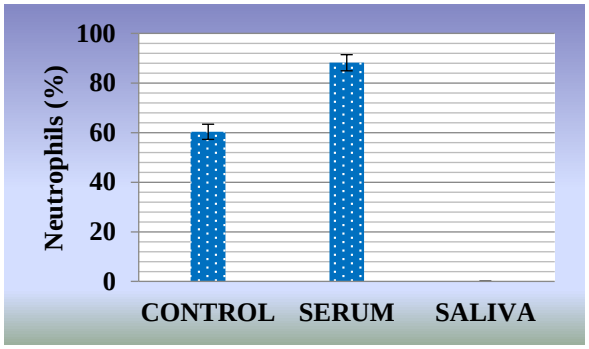

Fig. 7. Neutrophils % in serum and saliva.

DISCUSSION:

The variables performed, showed a significant difference in leukemic patients and controls hence proving the link of these variables to the occurrence and prevalence of leukemia. The study was performed on serum and saliva samples and the parameters included MDA, Isoprostanes, 8-hydroxydeoxyguanosine, tumor necrosis factor-alpha, myeloperoxidase, neutrophils, and interleukin 6. All of these variables were observed to be influenced by leukemic conditions as compared to control. An increase in the levels of MDA and other reported inflammatory markers in the serum samples signifies their importance, it shows MDA levels were increased with the elevation in the disease condition and led to increased DNA damage which was observed in the terms of increased levels of isoprostanes and 8-hydroxydeoxyguanosine.[12,13] And uptake of interleukin-6, a pro-inflammatory cytokine along with its regulatory actions in metabolism, regeneration, and neural processes. It provokes the immune and hematopoietic actions. Tumor necrosis factor is a multifunctional cytokine involved in many physiological processes that control inflammation, antitumor response, and homeostasis through its receptors. These receptors mediate cytotoxicity, T cell proliferation, and conflict with infection. Inflammatory cytokines play an important role in the onset and progress of hematological malignancies.[14]

Literature shows the role of TNF-alpha, IL-6, IL-8, and CRP as survival prognostic markers in chronic lymphocytic leukemia. These pro-inflammatory markers play an important role in the pathogenesis of chronic leukemia. In hematological malignancy TNF-alpha, IL-6 and IL-8 were recorded to be higher while CRP levels were significantly reduced. These results are accordance with results of our study, showing high TNF-alpha and IL-6 levels were non-significantly higher showing a high burden of disease. Therefore, proving TNF-alpha a persistence analytical marker in chronic lymphoid leukemia. Tumor necrosis factor is involved in interactions between a leukemic cell and normal BM cell which provide a suitable environment for leukocytes to survive. TNF can be produced by macrophages, NK cells, neutrophils, etc. There are conflicting reviews of TNF roles as it is supposed to be helping in tumor growth and according to some studies it initiates apoptosis of tumor cells. TNF was higher in patients of acute myeloid leukemia in accordance with our results.[15] According to a study by Kim *et al.*,[16] Myeloperoxidase serves as an important factor in distinguishing leukemic patients from the ones that need a transplant. Hence, all of the above-stated studies were in accordance with our results showing elevated levels of MDA, Isoprostanes, 8-hydroxydeoxy guanosine, IL-6, MPO, neutrophils, and TNF-alpha.

CONCLUSION:

The findings of the current study conclude the role of oxidative stress and reactive oxygen species in the initiation of infection and leading to the development of leukemia in patients. Increased levels of MDA, IsoP, and 8-OHdG signifies alleviated DNA damage and increased oxidative stress in the patients. Thus, it may be stated that the treatment of the subjects with the antioxidants can have a significant effect on leukemic patients than in healthier subjects.

CONFLICT OF INTEREST:

Authors declare no conflict of interests

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AUTHOR'S CONTRIBUTION:

RA: Conceived and presented idea
MSQ: Collection of data, carried out experiment, writing
ZK: Collection of data, editing
SW: Writing, developed theory and performed computation
SI: Writing, performed analytical calculations,
NF: Editing
AM: Data analysis

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Review Article

ALZHEIMER'S DISEASE (AD): MANAGING COGNITIVE IMPAIRMENTS AND BEHAVIORAL PROBLEMS

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ABSTRACT:
Alzheimer's disease being a common and multifactorial neurodegenerative disorder is one of the most challenging and emerging issues in clinical medicine these days. The current therapy includes anticholinesterases and NMDA antagonists - memantine only. Owing to the advancement in the knowledge of its pathophysiology, a lot of research is going on and many potential targets and alternative therapies including compounds acting on the pathological substrate of the disease have been proposed, which may be beneficial in prevention and treatment of this debilitating disease.

Key Words: Cognitive dysfunction, Therapeutics, Neurodegenerative disease, Problems behavioral

INTRODUCTION:

Alzheimer's disease (AD) is one of the major degenerative diseases affecting almost 35 million people globally. It is characterized by dementia: a persistent and progressive impairment in intellectual function, and at least one of the other cognitive deficits: apraxia, agnosia, aphasia and/or impaired executive function.[1] The disease may be of early-onset, occurring between 30-60 years of age whereas late-onset AD, after the age of 60 years, accounts for around 90% of cases. It's prevalence doubles every 5 years in the older population, reaching 30–50% at the age of 85.[2] The disease itself is becoming a slow pandemic and it is expected that by the year 2050, one person for every 85 individuals may have AD.[3] Almost all patients with AD are affected by neuropsychiatric symptoms at some point during their illness which includes depression occurring earlier in the course of disease followed by irritability, anxiety, aggression and delusions as the disease advances. Furthermore, behavioral problems such as hostility, sleep disturbances, and wandering have been identified.[4]

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Pathophysiology:

Amyloid Hypothesis: The pathological hallmarks of AD are extracellular amyloid plaques consisting of highly ordered fibrils of Amyloid Beta (Aβ) and intracellular neurofibrillary *tangles* composed of the microtubule-associated protein tau.[5] The mechanisms responsible for neuronal dysfunction and death may include direct impairment of synaptic transmission, oxidative stress, excitotoxicity and neuro-inflammation.[6]

Cholinergic Hypothesis: The most striking neurochemical disturbance in AD is a deficiency of Acetylcholine (Ach) due to atrophy and degeneration of subcortical cholinergic neurons which modulate cognition, learning, task, memory-related activities and maintain sleep-wake cycle as well.[7] AD, however, is a complex disorder and involves multiple neurotransmitters, including glutamate, serotonin, and neuropeptides.[8]

Genetic Relationship: Autosomal dominant AD is caused by mutations in the following three genes responsible for the formation of Aβ peptides: Amyloid Precursor Protein (APP), PSEN1 (Presenilin) and PSEN2.[9] β-secretase and γ-secretase generate Aβ by successive proteolytic cleavage of APP.[10]

Diagnosis:
For effective treatment, it is very important to get an early and accurate diagnosis of Alzheimer's disease. AD is diagnosed mainly clinically, based on the presence of memory impairment (especially short-term loss) and other cognitive impairments that are insidious, progressive, and not well explained by another disorder.[11]

Risk Factors.[12]

Non-Modifiable	Modifiable	Others
Age Genetics (APOE E_4, Presenilin) Down Syndrome (trisomy 21) Traumatic Brain Injury	Hypertension Diabetes Elevated Homocysteine, Cholesterol Environmental Factors (exposure to silicon, aluminum & other toxins, free-radicals, metals like Cu, Fe, Zn; etc.)	Inflammation Oxidative Stress Estrogens

Treatment:
The discovery of specific proteins that accumulate and aggregate in the AD has opened the door to new therapeutic approaches. To date, no approved therapy directly targets the disease proteins (Aβ, tau). However, there is intensive research going on to bring disease-modifying treatments into clinical care.[13] Many of the existing therapies are neurochemical, aiming to replace or compensate for damage to specific neurotransmitter systems that are selectively impaired.[14] The goal of the current review is to discuss possible therapies.

Symptomatic Treatment

i. Cholinesterase inhibitors (ChEIs). They constitute the current first-line therapy for symptomatic treatment of cognitive impairments in mild to moderate AD. The FDA-approved ChEIs used for AD are rivastigmine, galantamine, and donepezil. Their adverse effects have been attributed to excessive peripheral cholinergic stimulation.[15] Tacrine was approved by the FDA in 1993, but the extent of alanine aminotransferase elevation and hepatotoxicity limited its use.[16] Although these drugs are not curative and don't alter the pathology of AD whereas the magnitude of evidence demonstrates that they delay the deterioration in cognitive function, behavioral manifestations and thus improve the overall well-being of the patients.[17]

ii. Non-Competitive N-methyl-D-aspartate (NMDA) Antagonist: Memantine. It is either used as an adjunct or an alternative to anti-cholinesterases, generally in later stages of AD. Its long-term functional outcomes have yet to be demonstrated.[18]

Disease-Modifying Interventions

i. Cerebrolysin. It has neurotrophic effects similar to that of endogenous nerve growth factors, which may play a role in AD pathogenesis by preserving neuronal function.[19]
ii. Ferulic Acid. It is a new therapeutic agent, which inhibits the Aβ - aggregation in experimental models.[20]
iii. Posiphen. It may slow the onset of disease or delay its progression by inhibiting the production of APP.[21]
iv. Agmatine. It activates antioxidant signaling pathways and thus may be a promising agent for improving cognitive decline and attenuating apoptosis in AD.[22]
v. Aducanumab. It may be beneficial in early diagnosed disease, by preserving memory and improving skills that could slow the disease progression.[23]
vi. Tramiprosate. It is an anti-amyloid aggregation agent and may help to treat mild to moderate form of AD.[24]
vii. Tarenflurbil and Semagacestat. They decrease Aβ formation by inhibiting γ secretase and thus may delay the progression of AD.[25]

Invasive Therapies

i. Deep Brain Surgery (DBS). It modulates the neurobiological activity and

improves cognitive function in patients with AD.[26]

ii. Memory Prosthetics. An artificial hippocampal system implanted in the rats' brain restored long-term memory. These findings open up amazing possibilities for ameliorating brain damage caused by AD.[27]

iii. Transcranial Magnetic Stimulation. Studies have shown that repetitive transcranial magnetic stimulation of the prefrontal lobes produce a significant improvement in the patients' ability to understand spoken language.[28]

Non-Pharmacologic Strategies

Behavioral problems in patients with AD are often best managed non-pharmacologically. Communication with the patients should be in simple language and their daily activities must be broken down into simple component tasks. Concealing doorways and encouraging movement under supervision may limit wandering. Additionally, minimizing daytime naps, limiting bedtime, cognitive behavior therapy and bright light therapy may be beneficial to the patients having sleep disturbances.[29]

Pharmacologic Approaches

Pharmacologic treatment should be reserved for patients who pose an imminent danger to others or themselves or when symptoms are substantially distressing to the patient.

Pharmacological options

Atypical Antipsychotics. The atypical antipsychotic agents: olanzapine, quetiapine, risperidone, and aripiprazole are increasingly becoming the first choice for agitation and psychosis in AD because of their better safety profile compared to typical agents (haloperidol) but must be used with caution in patients with vascular risk factors due to an increased risk of stroke.[30]

Benzodiazepines can be used occasionally for acute agitation. However, their adverse effects on cognition don't make them a better choice for long-term management.[31]

Antidepressants. They are used to combat symptoms of agitation and depression in patients with AD. Citalopram (a SSRI) has shown promising effects in clinical trials.[32]

Cholinergic agonists. Tacrine also resulted in the reduction or stabilization of delusions and xanomeline resulted in a greater reduction in episodes of delusion, suspiciousness, fearfulness, agitation, or wandering than the placebo.[33]

Electroconvulsive therapy (ECT). ECT has been adopted for depression, agitation and psychosis due to AD, but is mainly reserved for life-threatening or pharmacologically-unresponsive conditions.[34]

Emerging Therapeutic Approaches/ Novel Research Targets.

Future trends include the use of multiple drugs acting by different mechanisms such as antioxidant and anti-inflammatory action and inhibiting the formation of ß-amyloid plaques and fibrillary tangles.

i. Omega-3 Fatty Acids: DHA and EPA; Natural antioxidants; vitamin D_3 and E; and phosphatidylserine (a phospholipid) play a pivotal role as modulators of neuronal function, cognition, immune response and oxidative stress mechanisms in the brain. Hence, may be beneficial in the prevention and treatment of AD.[35]

ii. Selegiline. It is a monoamine oxidase inhibitor with antioxidant properties.[36]

iii. GABAergic Modulation. Etazolate, a $GABA_A$ modulator, α-secretase and phosphodiesterase-4 inhibitor, was proved beneficial in a recent trial, but the effectiveness and long-term benefits are yet to be determined.[37]

iv. Serotonin Receptor Modulation. Many serotonergic drugs (MAOIs and SSRIs) are under consideration as monotherapy or with ChEIs for their cognitive enhancing capacities.[38]

v. Histaminergic Modulation. Selective H_3 antagonists have shown positive effects on attention and memory, but their therapeutic role is not clear yet.[39]

vi. Adenosine receptor modulation. In vivo studies have shown the neuroprotective role of an adenosine 2A blocker.[40]

Preventive Treatments

The anticipated rise in the vulnerability of an older population to AD has led to the consideration of preventive therapy that will require the development of safe treatments or interventions that could be used in a large number of susceptible individuals. [(2)] Non-steroidal anti-inflammatory drugs, estrogen-replacement therapy, and an anti-amyloid vaccine are a few potential preventive therapies under consideration.[41]

Alternative Therapy

Phytotherapy enhances the brain's ability to function, and therefore, provides stability when used consistently.

Neuroprotective Mechanisms of Plant Extracts.[42] - Cholinesterase Inhibition: Achyroclinetomentosa, Eupatorium viscidum, Ruprechtiaapetala, Zanthoxylum coco, Salvia officinalis, Trichoclinereptans, Angelica archangelica, Poncirus trifoliate, Treculiaobovoidea, Cassia obtisufolia, Desmodiumgangeticum, Huperzia Serrata. Modification of Monoamines: Moringa oleifera. Antioxidant activity: Desmodiumgangeticum, Ginkgo biloba, Salvia officinalis. Antiamyloid aggregation effect: Ginkgo Biloba.

Neuroprotective Effect of Traditional Plant Extracts.

Japanese-Chinese Medicines**:** Research demonstrated their probable axonal extension activity against amyloid β induced axonal atrophy; improving memory impairment.[43] European Plant extracts: S.triloba and Teucrium polium have shown effectiveness in managing mild to moderate AD by amelioration of cognition.[44] Ayurvedic Herbs: Ashwagandha, Shankhpushpi, Guggulu, Gotu Kola, Curcuma longa and Bacopa monnieri may help in improving the symptoms and progression of AD.[45]

Nutritional therapy. Studies in recent decades demonstrated the role of nutrition in treating dementia. Healthy dietary changes, in particular switching to a diet composed of whole grains, fish, nuts, fruits, vegetables, low-fat dairy, healthy oils, and eliminating white sugar reduce cognitive decline and prevent the early onset of AD. Although, its effectiveness varies from person to person but it's likely to be beneficial.[46]

Lifestyle Changes. Studies show that physical activity can slow down and even prevent the progression of cognitive decline in AD. Gardening, walking, yard work, and even dancing may help.[47]

Social Interaction: Psychosocial intervention is a great approach to improve cognition and overall wellness in patients with AD. There are many ways to improve the quality of life and possibly dementia symptoms through social activities such as talking about events from the past, taking part in group activities to improve memory, problem-solving skills, and doing everyday tasks.[48]

Acupuncture: Recent clinical trials have shown that not only is acupuncture a safe option that improves cognitive ability, but improves pain and insomnia too.[49]

Reflexology: Massages improve quality of life by reducing pain and distress in patients.[50] Aromatherapy: It has positive effects on the reduction of behavioral and psychiatric symptoms of AD, enhancement of cognitive functions and improving quality of life.[51]

Prognosis

Life expectancy after a diagnosis of AD is reported to be 3–15 years. Hospital care is usually preferable for patients with end-stage disease.[1]

CONCLUSION:

Alzheimer's disease (AD) is a multifactorial neuro-degenerative disorder. Although a lot of research and clinical trials are going on but despite all the scientific efforts, no pragmatic curative therapies have been found yet. The three anti-cholinesterases; donepezil, rivastigmine and galantamine along with memantine, constitute current mode of therapy. Additionally,

antipsychotics and antidepressants are used to ameliorate the behavioral problems associated with the disease. Treatments under research include compounds modifying the pathological substrates of the disease: Aβ, APP and tau protein.

Author's Contribution:

SA: Conception of work, design and supervision
MP: Acquisition of data and substantial contribution in design
MN: Drafting article
AF: Reference writing
SJ: Reviewing article critically

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Case Report

MORPHINE OVERDOSE IN A PATIENT USING PATIENT CONTROLLED ANALGESIA (PCA) - A CASE REPORT

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ABSTRACT:

Background: Good post-operative pain control is crucial to the success of the surgery and the well-being of the patient. Pain relief after surgery is the most important concern amongst patients. The term, PCA, refers to on-demand, periodic intravenous administration of opioids, which can be operated by the patient to administer self- medication. The use of PCA has improved control of pain in the immediate period after surgery. The use of these advanced machines with a high degree of complexity has led to the addition of new sources of errors including programming errors.

Case Description: A twenty-one years old male patient with a confirmed diagnosis of a soft tissue tumor "angiomatoid fibrous histiocytoma", was enlisted for excision of the tumor tissue with groin nodes clearance along with pedicled posterior tibial artery propeller flap. Initially, epidural was used for postoperative analgesia. However, due to continuous motor blockade, the decision was made to discontinue the epidural and use PCA morphine for controlling postoperative pain. Due to an error in setting up of the electronic device, 45 mg of morphine was administered to the patient instead of 1 mg bolus. After an interval of five minutes post-drug administration, the patient complained of nausea, headache, and vertigo.

Practical Implications: On duty nurse immediately alerted the anesthesia team about the morphine overdose. The patient was shifted to the surgical extended care unit for monitoring of the cardiac and respiratory function. The patient's haemodynamic parameters remained within the normal range and no airway intervention was required.

Recommendations: Based on this incident, regular training of the anesthesia trainees should be carried out and software of the PCA devices should be adjusted to minimize errors when complex parameters are entered.

Key Words: Analgesia, Surgery, Anesthesia

INTRODUCTION:

Inadequate pain relief in the post-operative period is associated with serious outcomes which can lead to hypertension, ischemia of the myocardium, ineffective deep breathing, delayed healing of the surgical wound, psychological disturbances, and development of long-term pain implications. Pain relief after surgery is the most common concern of every patient enlisted for a surgical procedure. Even though we fully appreciate the need for adequate postoperative analgesia, this area of perioperative care requires a lot of meticulous work to meet the desired standards.[1]

Patient-Controlled Analgesia (PCA) refers to on-demand, intermittent intravenous administration of opioids, which can be operated by the patient to administer self-medication. This technique enables a patient to administer the pre-programmed dose of medication by a simple click of a button attached to a computerized infusion pump.[2]

The use of PCA pumps has improved the level of patient comfort. The use of these devices is not without shortcomings, like medication errors and setting up errors. The input of inappropriate concentration of drugs, the volume of boluses, and lockout interval are a few examples. These errors can result in either inadequate or excessive

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administration of a drug, leading to fatal patient outcomes.[3]

Data from the US Food and Drug Administration (FDA's) Manufacturer and User Facility Device Experience (MAUDE) database showed that "operator errors were responsible for 6.5% whereas programming errors were associated with 81% of intravenous patient-controlled analgesia related complications. The other linked errors involve pump malfunction in the form of malfunctioning wires and damaged drug containers".[4]

CASE HISTORY:

A male patient, aged 21 years, with a confirmed diagnosis of a soft tissue tumor, "angiomatoid fibrous histiocytoma", was enlisted for excision of tumor tissue and clearance of the groin nodes along with posterior tibial artery propeller flap. Previously, the patient has had multiple uneventful surgeries under general anesthesia. There were no associated medical conditions. The patient's physical activity was reduced due to pain. Anesthesia plan for the procedure included general anesthesia and lumbar epidural. The course of the procedure remained unremarkable. After surgery, the patient was shifted to the post-anesthesia care unit (PACU) with an epidural infusion of 0.125% bupivacaine. Later the patient was transferred to the ward after stabilization.

On the following day, the patient complained of paresthesia and weakness in his left leg. The anesthesia team reviewed the patient and stopped the epidural infusion. Four hours later, the patient was again reviewed by the anesthesia team, at which time the motor loss was still persistent. At this point, it was decided to replace epidural with PCA morphine for postoperative pain relief.

An epidural catheter was removed and an electronic PCA device was set up by the anesthesia resident on call. Past midnight, the patient experienced pain for which the patient activated the PCA device as instructed. Following this, the patient received 45 ml of morphine rather than the desired volume of 1 ml. Five minutes post-infusion of morphine, symptoms of nausea, and vertigo were reported by the patient.

MANAGEMENT:

Anesthesia trainee immediately attended the patient and found the patient to be fully conscious having stable hemodynamics with a respiratory rate of 10 breaths/min. The patient was shifted to the surgical extended care unit (SECU) for monitoring. On later evaluations, the patient was found to be pain-free. Therefore, PCA morphine was discontinued and regular oral analgesics with intravenous boluses of morphine, as per need, were started.

An error in the setting up of the electronic PCA pump was identified as the cause of the unintended administration of a large dose of morphine to the patient. Subsequently, steps were taken to ensure that these mistakes are minimized. Re-education of anesthesia trainees was carried out. Along with this, biomedical engineers of the concerned device were contacted with the aim of resetting of the device software. The volume of the bolus that can be administered at one time was limited to 1 ml. Preset protocols were designed which can be used for each patient without the need to enter complex parameters. However, password-protected flexibility was provided to meet special requirements.

DISCUSSION:

The quality of care catered by any healthcare institution depends upon the consideration given to patient safety. Amongst medical errors, drug errors are the most common errors encountered. It includes errors during the administration of medications with the potential to cause fatal harm.[5,6]

All institutions, who offer PCA to their patients should have established policies regarding the administration of this service.[7] These policies should include: identification of appropriate patients, standardized order sets for drug orders, detailed documentation,

use of checklists, use of standardized pumps throughout the organization, reporting of adverse events if any along with details of appropriate monitoring.[8-10]

Monitoring the degree of sedation in patients using PCA is another important aspect of patient safety. Sedation monitoring scales are useful tools in the identification of those patients who may be overly sedated. In those patients, who are receiving supplemental oxygen, the use of capnography may be a more sensitive tool in identifying respiratory depression as compared to oxygen saturation alone. Some hospitals have also introduced bar codes for patients and medications which are dispensed from the pharmacy. These codes need to be matched before any medication is administered.[11,12]

These policies should also consider a pathway for the regular audit of the entire process and corrective actions to ensure delivery of safe and efficient services to the patients.[8-10]

CONCLUSION:

The Quality and safety of health care provided by any health organization to its patients depend upon the development and implementation of guidelines for clinical practice. These guidelines should be detailed, appropriately designed and pretested before their inclusion in the policies of a health care system.[7]

For ensuring safe and efficient delivery of PCA services, a task force consisting of all stakeholders i.e. members of acute pain services, nursing managers, line managers, pharmacy leadership, quality improvement, and any other members as deemed necessary, should be formulated. This task force should be charged with the responsibility of identification of the current need for provision PCA, a systematic review of current evidence, and formulation of transparent plans for implementation of the decided policies.

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MAB: Conception of work and design
AWK: Drafting and reviewing article

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