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Fetal Exposure to Risky Drugs: Analysis of Antenatal Clinic Prescriptions in a Nigerian Tertiary Care Hospital

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Abstract

Objective: To assess fetal outcomes after in-utero exposure to unsafe drugs.

Methods: This was a retrospective cohort study using data from medical records of pregnant women who received antenatal care over a two-year period (2019/2020). Inclusion was based on identification of prescription of potentially risky medications during pregnancy. Medication records, as well as delivery data, were extracted for analysis. The Australian drug evaluation committee classification system of risky medications was used for analysis.

Results: Results showed that 44–65% of medicines prescribed in pregnancy carry significant risks to fetal wellbeing. Fetal outcomes showed high levels of low birth weight, still birth, and early neonatal death. The common medicines prescribed irrationally in pregnancy were, among others, antibiotics, ACEIs, NSAIDs, Biguanides, and opiates, all of which are associated with adverse fetal outcomes.

Conclusion: There is a high level of fetal exposure to risky medications and adverse delivery outcomes. There is a need to improve prescription through prescriber training and awareness raising on existing guidelines on good prescribing practice for pregnant women.

Keywords: Drug prescriptions, fetal drug exposure, fetal wellbeing, low birth weight, pregnancy

Introduction

Pregnancy is a time of rapid fetal development and administration of drugs during pregnancy can carry the risks that could interfere with the organogenesis, structural growth, and other physiological processes of the fetus, which is particularly high in the first trimester of pregnancy. This is possible because many drugs can circulate freely in the maternal body and easily reach fetal circulation in concentrations that can cause major disruptions to development. In addition to the risk of disruptions to the fetal growth and development, drugs also have the potential to produce other subtle effects during post-delivery functional development of infants. There is literature evidence linking fetal drug exposure and learning difficulties, mental health disorders, obesity, organ dysfunction, and also a high risk of chronic diseases later in adult life.[1]

Fetal drug exposure is widely reported in high income countries; however data from low income countries is limited.[2] Recent studies in sub-Saharan African countries reported risky fetal exposure to antibiotics, analgesics, and antimalarial drugs.[3] The exposure, which is largely from prescriptions, is also exacerbated by the widespread self-medication practice among pregnant women as well as the general population.[4] One study reported that up to 80% of pregnant women may have used at least one risky drug through medical prescription or as over-the-counter medication.[5]

Among the most commonly reported fetal drug exposures that have been associated with various adverse outcomes are exposures to Benzodiazepines[5], Quinolones[6,7], antifungals,[8] Opioids,[9] NSAIDS,[10] Amlodipine,[11] and ACEIs/

ARBs.[12] Fetal exposure to certain types of drugs significantly increases the risk of low birth weight infant,[13] attention deficit problems in childhood, congenital malformations, as well as increased risk of childhood asthma.[14] The decision to prescribe drug(s) rests with the healthcare provider who is expected to follow the same principles of rational and safe use of drugs during pregnancy. The safety and audit of prescription medicines in pregnancy have received little research interest in this part of the country; therefore, this study aimed to audit prescription medicines given to pregnant women and also identify potential risks to fetal wellbeing.

Methods

This study was performed at the obstetrics and gynecology department of the University of Maiduguri teaching hospital, Nigeria. This was a retrospective study using medical records of women who received full antenatal care during a two-year period (January 2019–December 2020) from the hospital. The sample size was determined using the Andrew Fishers method and sample of medical records of five hundred women were selected using the simple random sampling method. Eligibility criteria included pregnant women who had received at least six months of antenatal care and also delivered in the hospital. Those who had missed more than half of their regular antenatal visits or were on antiretroviral therapy were not included in the study.

The data extracted from the medical records were of the subjects were demographic data, gravida, morbidity, potentially risky drugs, neonatal weight, neonatal deaths, stillbirth, APGAR score (5 minutes), and other relevant data. Data were entered into the SPSS version 21 for descriptive statistics, and fetal drug risk assessment was performed using the Australian drug evaluation committee classification or known as ADEC. The ethical approval for this study was obtained from the health research ethics committee of the University of Maiduguri teaching hospital Borno State, Nigeria.

Results

Demographic data showed that most pregnant women had secondary to tertiary education level (89%) with an average of 1 – 3 pregnancies (88%) per woman. Most of them presented themselves for their first antenatal care at 9 to 16 weeks of pregnancy (74.6%; Table 1).

Anemia was found to be common among pregnant women as reflected in the average pack cell volume of 30.4%, which was well below the recommended value. The results also showed that a quarter of them experienced pre-eclampsia (25.8%), while 4.6% experienced post-partum hemorrhage. There was a high incidence rate of low birth weight (64.8%), while stillbirths and early neonatal deaths occurred in 4% and 5.8% of pregnancies, respectively (Table 2).

The most frequent morbidities encountered were hypertension (53%), anemia (31.8%), and pre-eclampsia (25.8%). Other encountered diseases, albeit less frequently, were malaria, urinary tract infections, and also gestational hyperglycemia (Fig. 1).

Fetal exposure to potentially risky drugs was observed thoughout pregnancy with metronidazole (34.4%), Diclofenac (20.3%), and Captopril (21.1%) being most frequently

Table 1 Demographic Data

Variable	n (%)
Education	
Illiterate	2 (0.4)
Primary	53 (10.6)
Secondary	261 (52.2)
Tertiary	184 (36.8)
Occupation	
Civil service	139 (27.8)
Self-employed	169 (33.8)
Housewife	141 (28.2)
Student	51 (10.2)
Gravida	
1–3	440 (88)
4–6	51 (10.2)
>6	9 (1.8)
Average	2.4
Time of first antenatal visit (weeks)	
4–8	47 (9.4)
9–12	225 (45)
13–16	148 (29.6)
17–20	39 (7.8)
21–24	41 (8.2)
Mean (SD)	12.9 ± 4.4
Mean age (yrs.)	34.9 ± 7.4

Table 2 Maternal and Fetal Data

Variable	Result
Maternal data	
Packed cell volume	30.4±5.6
Bacteriuria	60.4%
Pre-eclampsia	25.8%
Post-natal hemorrhage	4.6%
Neonatal data	
Birth weight (kg)	2.6±0.7
APGAR score (5 minutes)	7.7±2.3
Still births	4%
Early neonatal death	5.8%

encountered. Other drugs of concern included Metformin, Levofloxacin, and Codeine, which were mostly prescribed during the third trimester of pregnancy (Fig. 2).

The prevalence of potentially risky drugs increased from 44.2% in the first trimester of pregnancy to 65.6% in the second trimester and then 60% in the third trimester. This represent an average of 56.6% of pregnancies had fetal exposure to risky drug (Fig. 3).

Based on the risk classification and risk description of drugs, it has been demonstrated that certain drugs, such as Captopril/Lisinopril (D), Diclofenac/Amlodipine and Metformin (C), Levofloxacin (B3), and Metronidazole (B2), pose various significant risks to the fetal organogenesis and development. In the case of Codeine-containing analgesics, despite the

Table 3 Prescription drugs and risk description

Drug	One	Two	Three	Classification	Risk description
Metronidazole	+	+	+	B2	Low birth weight, preterm delivery
Diclofenac	+	+	+	C	Premature closure of ductus arteriosus
Captopril/ Lisinopril	+	+		D	*Teratogenic*
Amlodipine	+	+	+	C	*Heart malformation, Hypospadia*
Diazepam					*Early motor deficit*
Metformin	+	+	+	C	*Low birth weight, high BMI, long term cardiometabolic disorders*
Hydralazine	+	+	+	C	*Low birth weight*
Gentamycin	+			B3	*Ototoxicity*
Levofloxacin	+	+	+	B3	*Arthropathy, preterm delivery*
Codeine	+	+	+	A	Neonatal respiratory depression

Notes: *+ = potentially risky, one, two and three = trimesters*

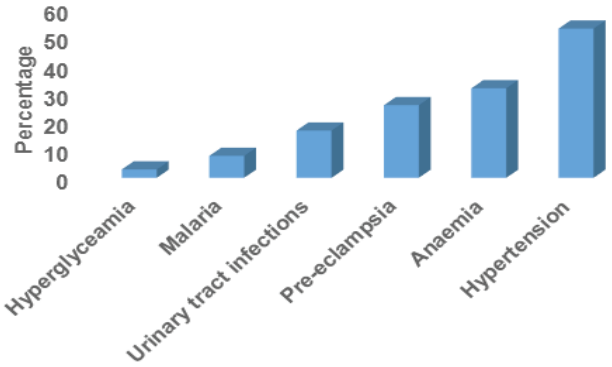

Fig. 1 Prevalence of Morbidities (n=500)

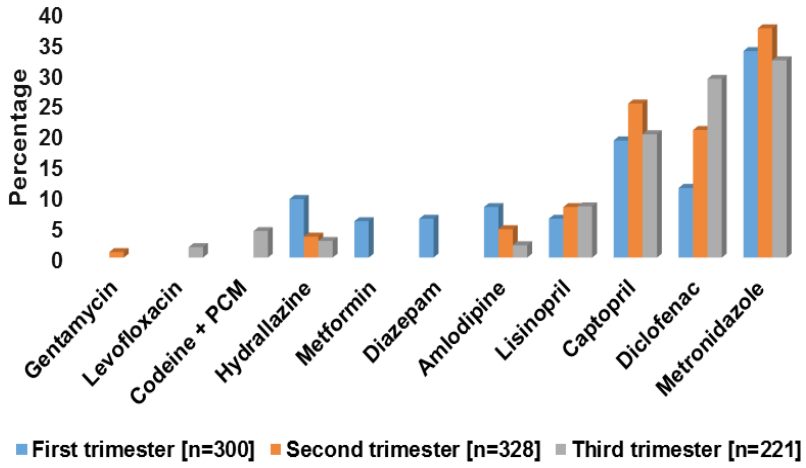

Fig. 2 Comparison of Fetal Exposure To Risky Drugs (n=500)

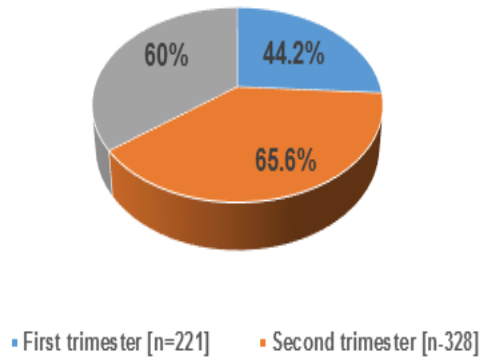

Fig. 3 Potentially Risky Prescription Drugs

fact that it may not pose a direct teratogenic risk, it carries significant risk for post-delivery respiratory depression with the potential for early neonatal death (Table 3).

Discussion

Fetal exposure to drugs cannot be completely avoided in pregnancy because of the need to manage maternal medical conditions. While general guidelines for drug safety in pregnancy are available, the decision for drug therapy rests with the physicians and other healthcare professionals provided care for these pregnant women. The results of this study showed that almost two thirds of pregnant women were prescribed drugs that are potentially risky to the fetal wellbeing. This result is comparable to a previous study,[15] although a similar study had reported much lower figure.[2] While there appeared to be more prescription drugs given as pregnancy progressed, there was a lack of due diligence on the fetal safety consideration on the part of prescribers. There are much safer alternatives to these risk drugs that are capable of producing comparable clinical outcomes. For instance, oral hypoglycemic drugs are contraindicated, with guidelines recommending a switch to the insulin therapy once pregnancy occurs. The risk of arthropathy associated with Quinolones, as well as cranial nerve damage with aminoglycosides and low birth weight with metronidazole has been reported and are well documented,[6] despite the existing contradictory conclusions on the fetal safety of metronidazole.

In addition to these known associations, antibiotics are well known to alter maternal and feto-placental microbiome,[16] which has been linked to childhood asthma.[17] They are also reported to cause altered fetal growth and childhood growth trajectory. [13,18] Some studies specifically reported increased risk of cerebral palsy and/or epilepsy with fetal exposure to macrolides.[14]

The recommendation for the treatment of hypertension in pregnancy has exluded the use of ACEIs because of their risk of teratogenicity; thus, the prescription of Captopril/Lisinopril is irrational when compared to the safer alternatives such as Methyldopa, Labetolol, and others that are also effective in achieving blood pressure control. In the case of Calcium

channel blockers, there is limited safety data, though, in recent years, Nifedipine has been considered safe in pregnancy. Nevertheless, some studies have reported the potential association between Calcium channel blockers and birth defects, such as heart malformation, hypospadias and low birth weight. [19,20]

The use of NSAIDs in pregnancy is not generally recommended because of their risk of premature closure of ductus arteriosus in the newborn and decreased neonatal renal function.[21,22] They have also been reported to carry the risk of low birth weight and development of asthma in infants.[5] Opioid use also carry a high risk of the occurrence of spina bifida among other congenital malformations.[9]

Metformin use in pregnancy exposes the fetus to the risk of higher body mass index in childhood and a significantly higher risk of cardiometabolic disorders later in adulthood. [23, 24] Benzodiazepines are not known to carry significant risk of congenital abnormalities; [12] however, some previous studies have linked them to childhood motor and communication deficits.[5, 25]

Although it is challenging to track the individual outcomes of fetal drug exposure, the high prevalence of low birth weight, still births, and early neonatal should be a matter of public health concern. It should also be acknowledged that a certain amount of the observed adverse fetal outcomes may be caused by other confounding variables acting alone or in conjunction with prescription drug exposure in utero. In conclusion, the prescription of contraindicated or unsafe drugs in pregnancy carries a significant safety risk to fetal wellbeing in utero and also to the post-delivery development. There should be improved awareness of safety guidelines and training of prescribers on safe use of drugs among pregnant women.

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Original Article

Adenosine Deaminase as Inflammatory Marker in Type II Diabetes Mellitus

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Abstract

Objective: To evaluate the enzymatic activity of adenosine deaminase (ADA) in type II diabetes mellitus (T2DM).

Methods: This study was conducted on 60 clinically diagnosed type II diabetes mellitus patients, with 60 healthy subjects as the control group. Subjects were enrolled in the study only after their written consent was obtained. The inclusion of diabetes mellitus cases (DM) was conducted as per the WHO guidelines. Estimation of enzymatic activity of serum ADA was performed by Kinetic method using a commercial kit.

Result: The observed serum ADA activity in DM patients was 48.34 ± 21.05 U/L, which was significantly higher in comparison to healthy controls (25.02 ± 5.78 U/L). The serum activity raised in about 80% of patients and they had higher values above the reference activity of 30 U/L. The increased activity of ADA among the diabetic subjects indicates inflammatory changes in these individuals.

Conclusion: It is possible that in the coming years, a new therapeutic strategy based on anti-inflammatory properties with beneficial effects on diabetic complications can be translated into real clinical treatments.

Keywords: Adenosine deaminase, inflammatory markers, type II diabetes mellitus

Introduction

Diabetes mellitus is a known chronic metabolic disorders characterized by the presence of hyperglycemia caused by the derangements in the metabolism of carbohydrates, lipids, and proteins. A large number of diabetes mellitus cases remains undiagnosed. This disorder is deemed incurable and persists life-long. Type II diabetes mellitus is considered as a lifestyle disorder, which develops due to a sedentary lifestyle, lack of physical activities, low intake of dietary fibers, etc. Type II diabetes mellitus can lead to morbidity and mortality through numerous microvascular and macrovascular changes. Various other risk factors that have significant contributions to the development of this disease include the environmental, genetical, and behavioral factors.[1]

In type II diabetes mellitus, alterations in the functions of the immune system is also observed. The T-cell mediated immunity is disturbed, leading to the impairment in the insulin responses.[2] This results in adverse changes such as the activation of leucocytes, elevated levels of cytokines in the circulation, as well as increased apoptosis. These changes suggest that inflammatory changes occur in diabetes mellitus. Various pro-inflammatory mediators are released from different tissues including activated leucocytes, adipocytes, and endothelial cells. The role of various biochemical products, i.e., Interleukin-6, C-reactive protein (CRP), Tumor Necrosis Factor (TNFα), leptin, and others have been studied widely and many researchers have proposed their potential involvement in inducing the pathogenesis of Type II diabetes mellitus. Also, some metabolic and inflammatory factors and their serum levels has also been shown to have

a direct effect on the amount of adipose tissues in the body.[3, 4]

Adenosine deaminase (ADA), a metabolic enzyme catalyzing deamination, has been shown to deaminate adenosine into inosine while also acting as a regulatory enzyme that maintains the extracellular and intracellular concentrations of adenosine.[5] ADA is an enzyme that is expressed ubiquitously and its activity is seen higher in tissues like thymus, GI tract, brain, and lymphoid tissues.[6] The enzymatic activity of ADA has been seen to be higher in lymphoid tissues and aids in increasing lymphocyte proliferation and differentiation. It has also been found as a producer of oxygen derived reactive oxygen species (ROS), as well as a stimulator for lipid peroxidation.[7] Adenosine acts through G protein-couples receptor and facilitates the normal cell physiology regulation in various tissues.[8] It also plays the role of a suppressant of inflammation for its ability to inhibit the inflammatory mechanisms, such as inhibiting T-cell activation and proliferation. Meanwhile, adenosine is supposed to mimic the activity of insulin on glucose and might somehow play a role in causing insulin resistance. Since, the concentration of adenosine is regulated by the activity of ADA, the insulin resistance can have ADA as the triggering factor. The increased ADA activity in diabetic subjects might be due to deranged insulin activity linked to the T-lymphocyte function.[9] This study aimed to evaluate the enzymatic activities of adenosine deaminase in type II diabetes mellitus and whether there is a possibility of an association between inflammatory and diabetic markers in this disease.

Methods

This study was a case-control study performed at the Teerthanker Mahaveer Medical College and Research Center in 2019 after receiving the approval from the Institutional Ethical Committee of the college under the ethical clearance number IEC/17-18/030. The study was conducted on 60 clinically diagnosed type II diabetes mellitus patients with 60 healthy subjects as the control group. Sample size calculation was performed using the statistical formula. All subjects aged between 30 and 45 years old and were only enrolled in the study after their written consent was obtained. The inclusion of diabetes mellitus cases was done according to the WHO guidelines. Individuals having a fasting plasma glucose level of ≥ 126 mg/dl or a random glucose level of ≥ 200 mg/dl on two occasions were included as cases in this study. Those individuals having inflammatory diseases like tuberculosis, cancer, gout, liver diseases, and kidney diseases were excluded from the study to rule out any increase in the ADA activities due to other inflammation conditions. Pregnant female subjects were also excluded. The estimation of enzymatic activity of serum ADA was performed using the Kinetic assay method with commercially available kits.[10] Data obtained were tabulated and analyzed using the SPSS.

Results

Table 1 and Table 2 describe the demographic distribution of the participant and the study parameter comparisons between subjects and control. As described in Table 1 and Fig. 2, the study parameter, i.e serum ADA activities as

Table 1 Demographic distribution of the study population

S.N	Subject	Male	Female	Total
1.	Healthy control	34	26	60
2.	Diabetes mellitus	37	23	60

Table 2 Comparison of Study Parameters between Diabetic Patients and Healthy Controls

Parameter	Type II Diabetes Mellitus	Healthy Control	p-value
Fasting Plasma Glucose (mg/dL)	178.96± 53.98	90.16 ± 12.23	<0.001*
HbAlc (%)	8.65 ± 1.83	5.26 ± 0.68	<0.05
Adenosine Deaminase (IU/L)	48.34 ± 21.05	25.02 ± 5.75	<0.001*

Table 3 Correlation of Serum ADA activity with Glycemic Status in Diabetic Individuals

Glycemic Status	r-value	p-value
Fasting Plasma Glucose	0.24	<0.05
HbAlc	0.38	<0.0.5

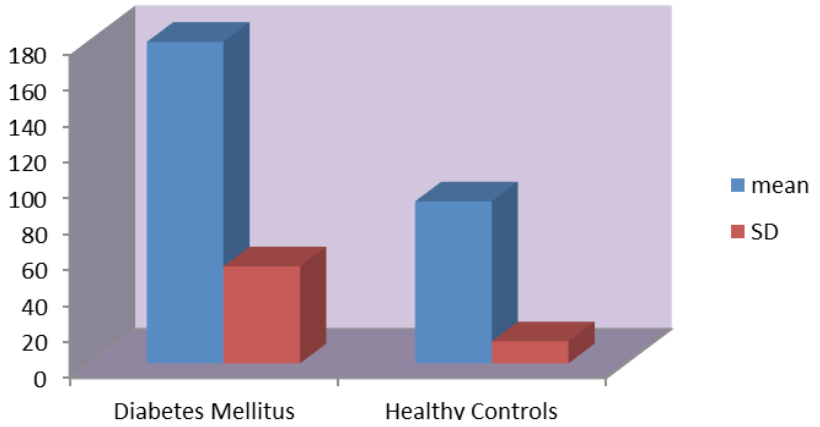

Fig. 1 Comparison of Fasting Plasma Glucose levels

one of the inflammatory markers, was found to be higher among type II diabetic subjects compared to the healthy subjects, and the difference in activity between the study groups was highly significant statistically ($p<0.01$). The correlation analysis between serum ADA activities and glycemic indices as shown in Table 2 also highlighted the fact that serum ADA activity was significantly correlated with fasting blood sugar ($p<0.05$) and HbA1c levels ($p<0.05$).

Discussion

Type II diabetes mellitus is a heterogeneous group of disorders that is featured by impaired insulin secretion and insulin resistance, as well as increased blood glucose. This disorder is supposed to be associated with an acute phase reaction, suggesting that a low-grade inflammation might also be involved in its pathogenesis.[11] Serum adenosine deaminase activity in this study was found to be elevated

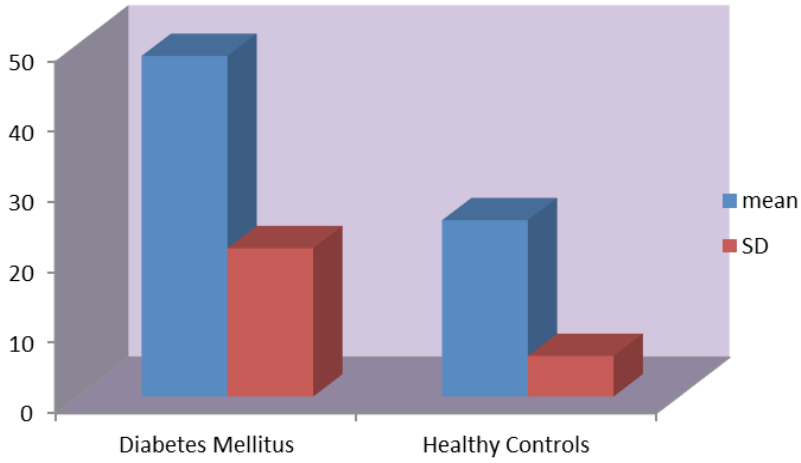

Fig. 2 Comparison of Adenosine Deaminase Activity

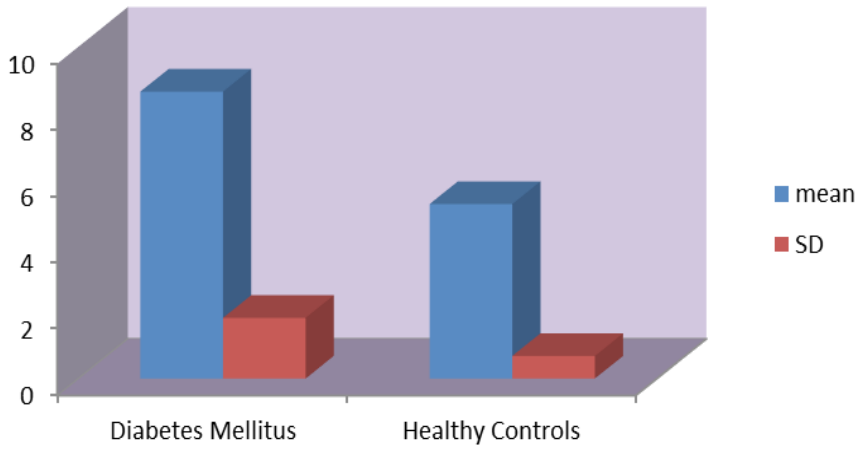

Fig. 3 Comparison of Glycosylated Hemoglobin Level

among the type II diabetes mellitus patients. On comparing the mean values using Student's t-test between the study groups, the serum activity of ADA in diabetic patients was seen to be 48.34 ± 21.05 U/L, which was significantly higher than that of healthy controls of 25.02 ± 5.78 U/L, as shown in Table 2 and Fig. 2. The serum activity was raised in about 80% of the patients and the value was higher than the reference activity, i.e., up to 30 U/L. An attempt to analyze the relationship of ADA as an inflammatory marker with the glycemic status of an individual was also made in the current study by applying the Karl Pearson's correlation coefficient. As a result, a positive and statistically significant correlation was observed with r= 0.385 ($p< 0.05$).

Several previous studies have reported an altered Serum ADA activity, which is quite variable. Kurtul N *et al* have shown increased level of serum ADA activity in diabetic patients and its correlation with HbA1c, and suggested that ADA might be an important enzyme for modulating the bioactivity of insulin effect and glycemic control. ADA may serve as an immune-enzyme marker in the etiopathology of type II diabetes mellitus according to some researchers. A study by Gitanjali G *et al* have reported an elevated serum activity of ADA and concluded that higher blood glucose levels aggravate the oxidative stress as well as raises ADA activity, which may be due to the local insulin resistance in the target organs. In the present study, since the serum activity of ADA was significantly highest among diabetic cases in comparison to non-Diabetic subjects, it can be suggested that ADA is an effective marker for inflammation in the case of type II diabetes mellitus.

Adenosine deaminase plays an important role in the proliferation and differentiation of lymphocytes, especially for T-lymphocytes. A higher activity of ADA is due to deranged T-lymphocyte responses, pointing towards the mechanism to release ADA into the circulation.[12] It is also speculated that an altered insulin related T-lymphocyte function could be the reason for the increased ADA activity in diabetes mellitus. Also, with the role of adenosine in the inhibition of lipolysis through the A1 receptors, as well as due to increased adenosine deaminase, the inactivation of adenosine and activation of lipolysis are observed .[13,14] This markedly potentiates the increment in the cAMP accumulation via nor-epinephrine action. Thus, deregulated lipid metabolism and consequent elevation of free fatty acids might lead to the pathogenesis of the type II diabetes mellitus.[15, 16] In this study, it was found that the activity of ADA was higher in diabetic patients. Meanwhile, this particular inflammatory marker was also found to be positively correlated with the glycemic status of a diabetic patient. Furthermore, the Pearson's correlation coefficient results showed a significant and positive correlation with the HbA1c levels of these patients. The positive significant correlation between serum ADA activity with the short term and long-term glycemic control indicates the important role of ADA in glucose and lipid metabolism derangements seen in type II diabetes mellitus. Nevertheless, this present study was limited in a way that only ADA is included as a

marker for inflammation, and a small sample size also have weaken the findings of the study. A large extended prospective study at the molecular level is suggested to explore the pathophysiological processes that are involved in the mediation of inflammation in diabetes mellitus circulatory levels that underlie the inflammatory mediators correlation with insulin resistance and the significant increase in groups at risk of type II diabetes mellitus.

This study has shown that inflammatory marker like ADA is linked to the development of insulin resistance and progression to diabetes mellitus type II. It is possible that in the coming years, the hope of new therapeutic strategies based on anti-inflammatory properties with beneficial effects on diabetic complications can be translated into real clinical treatments. The present study was limited to the sample size and a larger sample size studies would help in extrapolating the inflammatory role of ADA in T2DM. The role of several other inflammatory markers like hs-CRP, Interleukins, etc. in metabolic disorders would be an open area for future studies.

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Original Article

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Secondary Seizures in Pediatric Population in Two Tertiary Hospitals in India

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Abstract

Objective: To evaluate the clinical pattern of secondary seizures which includes acute and remote symptomatic seizures among hospitalized patients in two healthcare centers and assess the outcomes among hospitalized patients having secondary seizures.

Methods: This multicentric cross-sectional study was conducted in two tertiary hospitals in Odisha and Tamil Nadu, India, for a period of four years. A total of 274 patients in the age group between 6 months to 12 years participated in the study. A structured proforma was used to document the clinical pattern and causes of the secondary seizures.

Results: Among the participants in Odisha and Tamil Nadu hospitals, focal seizures constituted 67.5%. Generalized seizures were present in 32.4%. The key causes of seizures in Odisha were malaria, cerebral palsy, and viral meningitis, while in Tamil Nadu, the causes were neurocysticercosis, cerebral palsy, and viral meningitis.

Conclusion: Since the majority of the causes are preventable, it is important to address the issue at the public health level by providing improved sanitation and adequate awareness on the secondary seizure and its causes. It is also important that the physicians are well conversant with early case detection and treatment of primary diseases causing secondary seizures.

Keywords: Convulsion, encephalitis, malaria, secondary seizures

Introduction

Various causes has been attributed to seizures and it is currently well recognized that seizure is a symptom and not a disease. A seizure is defined as transient occurrence of signs and symptoms, resulting from abnormal, excessive, or synchronous neuronal activities in the brain. The definition from the World Health Organization (WHO) for secondary seizures describes them as seizures with an unknown underlying cause. Secondary seizures in this study include those conventionally referred to as acute symptomatic seizures and remote symptomatic epilepsy. Incidence of seizures during the first five years of life is about 5%, and around 4-10% of all children experienced at least one seizure episode in the first 16 years of life. The prevalence of childhood seizures varies between 5.2–8.1 per 1,000 population. The most common type (60%) of seizures are convulsive, where two-thirds begin as focal seizures that may then become generalized, while one-third of the cases begin as generalized seizures. The remaining 40% of the seizures are non-convulsive.[1]

Although seizures have been classified in many ways according to their site of origin, electroencephalogram (EEG) abnormalities, types of seizures, and response to therapy, a broad-based classification developed by Gastaut is applicable globally.[2] Furthermore,

a broad classification of epilepsy has been provided by the International League Against Epilepsy (ILAE) 2017 as focal, generalized, and unknown onset, with additional etiological classification that includes structural, genetic, infectious, metabolic, immune, and unknown etiology. Seizures with unknown causes are presumed to be of genetic in origin, while symptomatic seizures, either of genetic causes or acquired causes like hippocampal sclerosis, perinatal and infantile causes, cerebral trauma, tumor or cerebrovascular causes, provoke seizures secondary to provoked factors, and are cryptogenic in nature with presumed symptomatic nature in which the cause has not been identified.[3]

Seizures are considered as one of the non-specific symptoms expressed in a variety of different pathologies. In the majority of cases, the seizure phenomenology depends on the cortical location of the lesion, rather than on specific population. In addition, an early recognition of treatable causes of this common neurologic symptom and the institution of proper and adequate treatment will ensure normal physical, mental and psychological development of the child.[4] In terms of clinical patterns of secondary seizures, identifying a single underlying pathology of the seizure can be challenging. A brain tumor may produce seizures that are similar to other intracranial pathologies, e.g., infections and birth injury, and the treatment will be incomplete unless there is a correlation between the clinical and pathological aspects of seizures. As far as possible, the underlying pathology should be investigated for better management of secondary seizure disorders.[5] The present study was carried out to evaluate the clinic pathological profile of secondary seizures in pediatric population and to assess the outcomes of hospitalized pediatic patients with secondary seizures.

Methods

The present study was carried out as a multi-centric cross sectional study in two tertiary care teaching hospitals with a total bed capacity of 1,000 in Odisha and Tamil Nadu, respectively. The study was conducted for a period of four years of October 2012-2014 and Nov 2016-2018. Children in the age group of 6 months to 12 years were taken up for the study. Children with generalized convulsion with or without neurological deficit, clearly appreciable focal seizures with or without neurological deficit, intermittent quivering movements affecting one or more extremities with altered sensorium, facial grimace with altered sensorium, staring look with altered sensorium, chewing and sucking motion with sensorium deficit and slight posturing or barely perceptible tremor with disturbance in tone and reflex activity, and irregular movements with constant crying and irritability with known definite underlying cause for seizures were included in this study. All cases were included on the basis of history and examination performed by a senior pediatric consultant or pediatric neurologist. Idiopathic generalized epilepsy and conditions mimicking seizure disorders like apneic spells, tremors, and syncopal attacks were excluded. Approval was obtained from the Institutional Ethics Committee prior to the data collection. Detailed explanation on the study was given to each participant's parent or guardian and informed consent was obtained from the parent prior to the data collection. All pediatric patients who were admitted to the respective hospitals during the study period were taken purposively as the subjects of this study. A total of 264 patients participated: 168 patients participated in Odisha and 96 participated in Tamil Nadu. A structured proforma was used to obtain the history and clinical information regarding the subjects. Particulars regarding the course in the hospital, laboratory parameters, and outcomes were also documented. Data were entered into and analyzed using the SPSS ver.20 software. Descriptive statistics were used and the comparison of seizure profile with risk factors was evaluated using the chi-square test, with a p-value of <0.05 considered statistically significant. Ethical approval for this study has been granted by the health research ethics committee from the Communication of Decision of the Instuitional Ethics Committee (IEC)/Institutional Review Board (IRB), Indian Council of Medical Research, under the IEC/IRB:-43/12.

Results

Most subjects were in the age group of 3.6–7 years in both teaching hospitals (41.6%). Female gender constituted the majority of the population in Odisha and Tamil Nadu (57.2%). The majority of subjects in both locations belonged to a lower socioeconomic status and lived in rural areas. The most common type of seizures in studied cases were partial seizures, which were seen in 185 (67.51%) cases, which was followed by generalized seizures (32.4%).

Table 1 Background Characteristics and Type of Seizures

Demographic Details and Type of Seizures	Characteristics	Odisha & Tamil Nadu	
		n	%
Age (in years)	0.6–3.5	53	19.3
	3.6–7	114	41.60
	7.1–10.5	60	21.8
	10.6–12	35	12.7
Sex	Males	107	39.05
	Females	157	57.2
Socioeconomic status	Lower	137	50
	Middle	73	26.6
	Upper	54	19.7
Location	Urban	101	36.8
	Rural	163	59.4
Type Of Seizures	Generalized	89	33.71%
	GTCS	50	18.94%
	Tonic	22	8.33%
	Atonic	6	2.27%
	Clonic	9	3.41%
	Myoclonus	2	0.76%
	Focal	175	66.29%
	Aware	76	28.79%
	Impaired awareness	25	9.47%
	Focal to bilateral tonic Clonic	74	28.03%

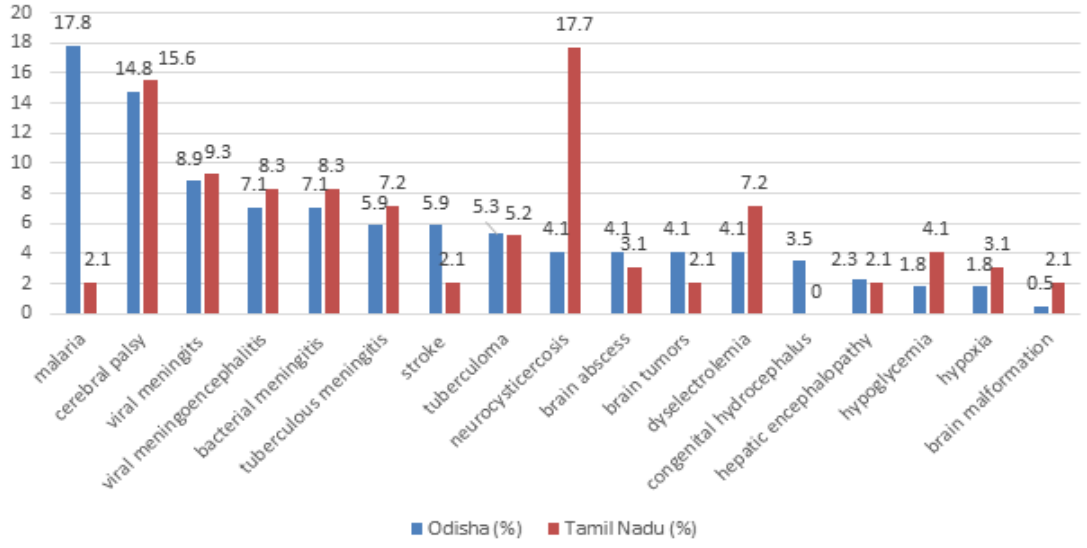

Fig. 1 Causes of seizures in Odisha and Tamil Nadu

Table 2 Clinical Presentation of Infective Causes

Symptom	No of cases (%)	Sign	No of cases (%)
Malaria			
Fever	29 (90.6)	Pallor	11 (34.3)
Lassitude	26 (81.2)	Icterus	5 (15.6)
Convulsions	32 (100)	hepatosplenomegaly	8 (25)
Altered sensorium	17 (53.1)	Meningeal signs	3 (9.3)
Decreased urination	11 (34.3)	Papilledema	4 (12.5)
Viral Meningitis			
Fever	22 (91.6)	Meningeal signs	19 (79.1)
Convulsions	24 (100)	Papilledema	8 (33.3)
Viral Meningoencephalitis			
Fever	8 (40)	Meningeal signs	5 (25)
Altered sensorium	18 (90)	Papilledema	3 (15)
Convulsions	20 (100)		
Loose stools	7 (35)		
Vomiting	4 (20)		
Bacterial meningitis			
Fever	14(70)	Meningeal signs	17 (85)
Altered sensorium	8 (40)	Papilledema	10 (25)
Convulsions	20(100)		
Neurocysticercosis			
Fever	2 (8)	Meningeal signs	5 (20.8)
Altered sensorium	7 (29.1)	Papilledema	3 (12.5)
Convulsions	24 (100)		
Brain Abscess			
Fever	7 (70)	Meningeal signs	8 (80)
Altered sensorium	7 (70)	Papilledema	5 (50)
Convulsions	10 (100)		

Amongst those with partial seizures, simple partial seizures were most common and were seen in 67 (27.7%) cases, whereas amongst those with generalized seizures generalized tonic-clonic seizures were the most common and seen in 50 (18.94 %) patients (Table 1).

The predominant causes of the seizures in Odisha were malaria, cerebral palsy, and viral meningitis, while in Tamil Nadu, the predominant causes were neurocysticercosis, cerebral palsy and viral meningitis. (Fig. 1).

From the clinical presentation for infective causes, it was demonstrated that fever was the most important symptoms and meningeal signs were the most important signs for most of the infective etiology. Convulsion being the inclusion criteria were seen in all cases (Table 2).

For the congenital etiology, the variation in head size either microcepahly or macrocepahly was the predominant sign. For those with metabolic etiologies, convulsions and altered sensorium were the dominant symptoms and most of the cases manifested with generalized systemic signs (Table 3).

The majority of the diagnosis in Odisha was based on CT scan, while the MRI scan is predominantly used in Tamil Nadu. The other investigations which were done to establish the etiology of seizures were complete blood count, rapid malarial antigen test, serum electrolyte test, hepatic and renal function tests, random blood sugar levels, and CSF examination in selected cases. The outcomes of various causes of secondary seizures was determined by using electroencephalograms

Table 3 Clinical Presentation of Congenital, Metabolic, and Other Causes

Clinical Presentation		Symptom	Odisha & Tamil Nadu n (%)	Sign	Odisha & Tamil Nadu n (%)
Congenital Causes	Cerebral palsy (n=40)	Convulsions	40(100)	Microcephaly	40 (100)
		Fever	5 (12.5)	Increased tone	31 (77.5)
		Tonic posturing	27 (67.5)	Hepatosplenomegaly	0 (0)
	Brain malformation (n=3)	Convulsions	3 (100)	Microcephaly	3(100)
		Increased tone	2 (66.6)	Hypertonia	2 (66.6)
	Congenital hydrocephalus (n=6)	Convulsions	6 (100)	Macrocephaly	6 (100)
		Increased head size	6 (100)	Macewens and transillumination	6 (100)
		Altered sensorium	6 (100)	Papilledema	3 (50)
Metabolic causes	Electrolyte Imbalance (n=14)	Convulsions	14 (100)	Signs of dehydration (sunken eyes, delayed skin pinch, dry mucosa)	5 (35.7)
		Altered sensorium	14 (100)		
		Loose stools	14 (100)		
		Vomiting	7 (50)		
		Decreased urination	6 (42.8)		
	Hypoglycemia (n=7)	Convulsions	7 (100)	Convulsions	7 (100)
		Altered sensorium	7 (100)	Altered sensorium	7 (100)
	Hypoxia (n=6)	Convulsions	6 (100)	Crepitations on auscultation	6 (100)
		Altered sensorium	6 (100)	Tender hepatosplenomegaly	5 (83.3)
		Hurried breathing	6 (100)		
		Fever	4 (66.6)		
Other Causes	Stroke (n=12)	Weakness of body and limbs	12 (100)	Hemiplegia	10 (83.3)
		Convulsions	12 (100)	Facial palsy	10 (83.3)
	Tuberculoma (n=14)	Convulsions	14 (100)	Meningeal signs	3 (21.4)
		Fever	4 (28.5)	Papilledema	3 (21.4)
		Altered sensorium	7 (50)		
	Brain tumors (n=9)	Convulsions	9 (100)	Meningeal signs	6 (66.6)
		Altered sensorium	6 (66.6)	Papilledema	3 (33.3)
	Organ failure (n=7)	Convulsions	7 (100)	Icterus	7 (100)
		Altered sensorium	7 (100)	Meningeal signs	2 (28.5)
		Yellowish discoloration of body	7 (100)	Papilledema	2 (28.5)

in three successive follow up visits in 3 month interval [3, 6 and 9 months] after the discharge. Although significant drop outs of more than 50% of patients were observed, there was an overall significant improvement of outcome, i.e., seizure free or seizure reduction along with EEG normalization, during the follow up period based on the evaluation using the EEG in both the centers. The outcome was better in children with acute causes of secondary

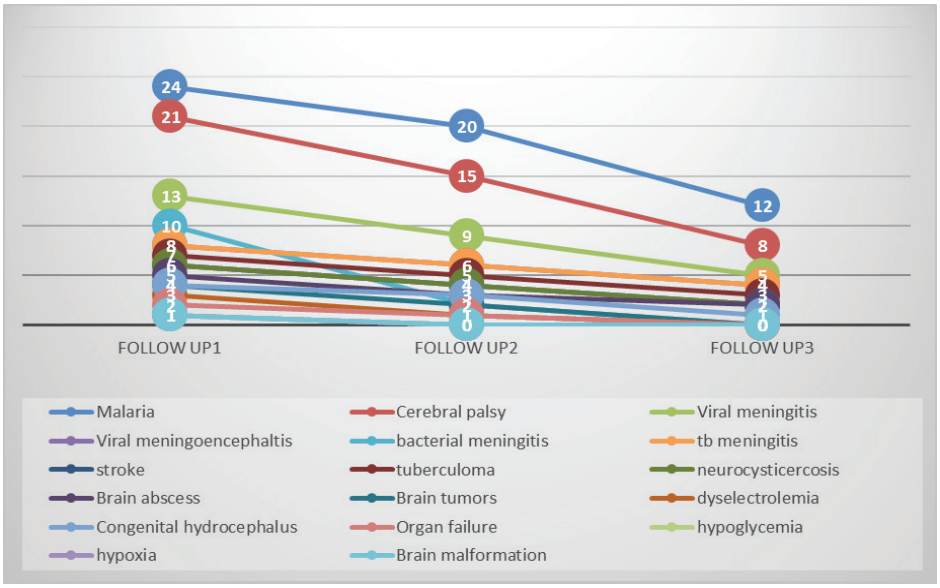

Fig. 2 Outcomes During Follow Up (Odisha)

seizures and also in causes in where no permanent brain injury was seen compared to remote symptomatic causes like cerebral palsy (Fig. 2 and Fig. 3).

Discussion

Secondary seizures are rampantly increasing in developing countries, including in India. However, there is considerable paucity in the availability of literature. This study was performed as a multicentric study of Odisha and Tamil Nadu on 168 and 96 patients, respectively. In this study, the incidence of seizures was more in early childhood between the ages of 3.5 to 7 years in both centers. Similar findings were observed in a study done by Kotsopoulos *et al*. and Neubauer *et al*. [4, 5] Therefore, the need for early detection of the symptomatology is high among younger

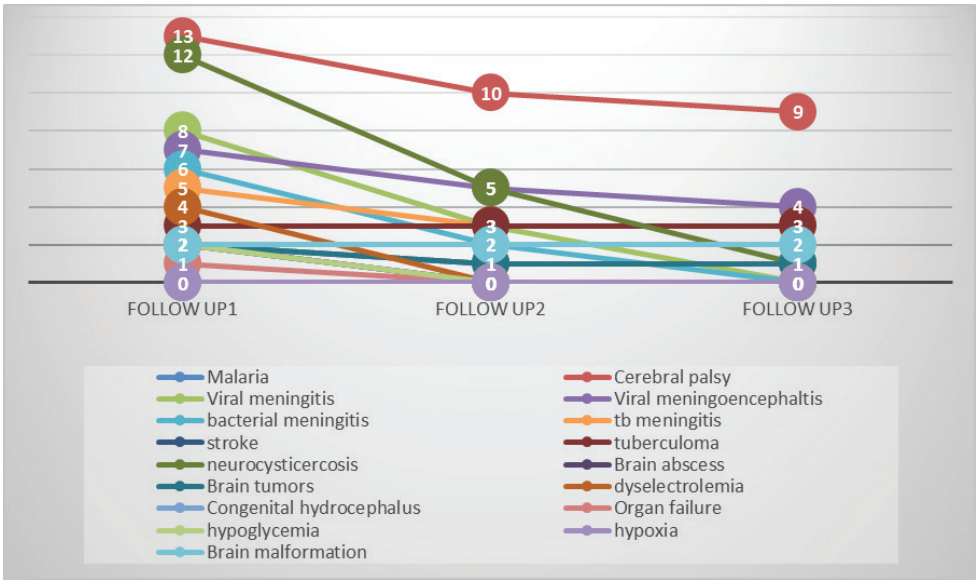

Fig 3 Outcomes During Follow Up (Tamil Nadu)

children. The majority of the subjects were diagnosed with partial seizures (71.5% in Odisha and 57.2% in Tamil Nadu). The findings are similar to the studies done by Unver *et al*.[6], where the prevalence of partial seizures is 56.5%.

The dominant causes of seizures among the study population are infective cause, essentially parasitic infections, with malaria as the strongest cause for seizures in Odisha (17.8%) while neurocysticercosis was the most common cause in Tamil Nadu (17.7%). Symptomatic infective etiology was present in 47.1% of the subjects. In a review done by Vezzani *et al*.[8], infections of the Central nervous system constituted the predominant cause for seizures similar to this study.[7] In another study done by Gowda *et al*., the predominant cause for seizures was structural abnormalities, while TORCH infections contribute 7.2% of the seizures. The reason for the high prevalence of malaria induced seizures in Odisha is due to the epidemiological vulnerability of the state. Odisha has several rural and tribal districts that are endemic to falciparum malaria. In a study done by Das *et al*.[9], the prevalence of cerebral malaria was found to be 18.5% among pediatric population in Odisha and 44.4% of the deaths in the pediatric age group was attributed to seizures in malaria. There has been a steady rise in the prevalence of seizures associated with neurocysticercosis in recent years. In various studies carried out in South India, the incidence of seizures was as as high as 94.8% among children with neurocysticercosis. This has been largely attributed to the changing lifestyle patterns resulting in formation of solid cystic granuloma by the Taenia solium parasite. [10]

According to a study by Nelson *et al*. the incidence of nonfebrile convulsions was highest in the first year of life, especially in the first month. Children with neurological or developmental abnormality assessed in the first year of life did not have their first seizure earlier than children without any abnormality. Neurological abnormalities in the first year of life before any seizure, and the presence of minor motor seizures, are associated with an increased rate of mental retardation and cerebral palsy at age of seven, but early age at onset appears to have little prognostic value regarding intellectual function, cerebral palsy, and epilepsy.[11]

Malaria and neurocysticercosis were the most common infective pathologies seen in studied cases. In various developing countries infective pathologies remain common cause of secondary seizures. Other than infective causes, non-infective causes such as cerebral palsy and dyselectrolytemia were the common causes of secondary seizures in these children.

Based on the study findings, it may be considered that secondary seizures are not recurrent, unlike primary idiopathic seizures, when the underlying cause is treated properly. Recurrent secondary seizures are observed in patients with residual brain damages due to primary disease or due to persisting primary disease and these children have much poorer outcomes. Age of onset and cause of secondary seizures have found to have important effect on the outcome of secondary seizures so our study is not compatible with their study since the trend secondary seizures is different from primary idiopathic seizures.[12]

This study is limited in terms of a relatively small number of patients; thus, a study with a larger number of pediatric patients will further substantiate the findings of this study. Moreover, in this study, neuroimaging such as computerized tomography or MR imaging could not be done in all cases. The present study has emphasized on the role of infectious etiology, specifically malaria as a cause of secondary seizures. Majority of the causes of secondary seizures in pediatric age group in our study were found to be either preventable or treatable. Precise etiological diagnosis will help in proper management of children having secondary seizures.

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Original Article

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Autoantibody Profile and Thorax HRCT Scan in Systemic Sclerosis with Restrictive Lung Disease

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Abstract

Objective: To identify auto-antibodies in systemic sclerosis with interstitial lung disease (ILD).

Method: This was a descriptive categorical study on auto-antibody profile in systemic sclerosis patients visiting the Rheumatology Clinic of Dr. Hasan Sadikin General Hospital, West Java, and Bandung during the period of January 2018 to December 2019 who were registered in the West Java Systemic Sclerosis Registry. Auto-antibody identification was performed using the Euroline immunoblot assays.

Results: Thirty six cases were identified during the study period with most of the cases involved women (n=35, 97.2%). The average age of patients participating in this study was 40 years, with an average duration of disease of 18 months. Diffuse cutaneous systemic sclerosis was found in 22 (61.1%) cases and limited cutaneous systemic sclerosis was observed in 14 (38.9%) cases. Specific autoantibodies were positive in 33 (91.6%) cases, with anti-topoisomerase I as the largest group, positive in 22 (52.9.3%) cases. This was followed by anti-Th/To in eight (15.7%) cases; anti-Ro52 in four (7.8%) cases; anti-centromere in three (5.9%) cases; anti-RNA polymerase in three (5.9%) cases; anti-fibrillarin in three (5.9%) cases; anti-Ku in two (3.9%) cases; and anti-PDGF in one (2.0%) case. High-resolution computed tomography of the lung showed 34 (94.4%) cases with ILD and 22 (61.1%) cases with severe lung fibrosis. Usual interstitial pneumonia was seen in 19 (52.8%) cases and non-specific interstitial pneumonia in 15 (41.7%) cases.

Conclusion: Anti-topoisomerase I, anti-Th/To, and anti-Ro52 are the most common autoantibodies observed in systemic sclerosis patients with ILD as the most prevalent feature detected with lung HRCT.

Keywords: Autoantibodies, diffuse cutaneous, lung fibrosis, restrictive lung disease, systemic sclerosis

Introduction

Interstitial lung disease is the leading cause of death among SSc patients.[1-2] Early diagnosis and assessment of the organs involved in this disease, especially the lungs, is crucial as they will lead to early treatment that will affect the morbidity, mortality, and quality of life of these patients.[1-3] Unfortunately, early diagnosis of systemic sclerosis is challenging. Many patients come to the hospital with advanced disease or visceral organ involvement. Early diagnosis of organ involvement in the early stages will have implications for more aggressive treatment.

Immunosuppressants, such as mycophenolate sodium and cyclophosphamide, given in the early stage of the disease will decrease the disease progression as seen with decreased autoantibody titer. Intravenous chemotherapy, such as cyclophosphamide, is usually given to SSc patients with interstitial lung disease and delays in this therapy will increase morbidity and mortality. Thorax HRCT scan is the gold standard for diagnosis of ILD, especially for the early-stage disease.[4,5] Due to its rapid progression, the usual interstitial pneumonia is associated with a worse prognosis. During screening, the pulmonary function test using the spirometry is usually used and it often shows a restrictive lung disease that should be viewed cautiously as extrapulmonary factors may influence the result.[6-7] The autoantibody testing has also become the gold standard for the detection of autoimmune disease and is often associated with disease progression.

Early assessment of autoantibody testing will benefit patients, especially in the early diagnosis of SSc with organ involment. Also, autoantibody testing has become the gold standard for detecting autoimmune disease and may contribute to the diagnosis of SSc and organs involved, especially the lungs.[8,9] Autoantibody titers are often associated with disease progression. A previous study has concluded that the SSc specific-autoantibody is associated with organ involvement. [9] For example, Anti-topoisomerase I, anti-U11/U12, anti-Ro52, and anti Th/To are associated with lung involvement. Anti-centromere is already proven to be associated with cardiovascular involvement (PAH). Meanwhile, anti-RNA polymerase is shown to have an association with cardiovascular (PAH) and kidney (acute renal crisis) involvement. In addition, anti-fibrillation is associated with cardiovascular involvement (PAH), as well as with musculo-skeletal injuries (myositis). An association between autoantibody subtype and clinical manifestation along with organ involvement is also observed. Severe manifestation is found in SSc with lung involvement; thus patients will seek treatment earlier. To date, there are no specific clinical markers for lung involvement. Therefore, it is expected that, in the future, the SSc specific autoantibodies testing may be used as a modality for organ involvement diagnosis, especially for detecting SSc patients with interstitial lung disease in early stages.[7,8]

Methods

This was a cross-sectional descriptive study performed on data collected from the West Java Systemic Sclerosis Registry. Diffuse and limited SSc outpatients visiting the Rheumatology Clinic of Dr. Hasan Sadikin General Hospital Bandung, Indonesia, and received treatment during the period of January 2018 to December 2019, aged over 18 years old, were included in this study. Systemic sclerosis patients with overlap syndrome with other rheumatic autoimmune diseases and incomplete medical records were excluded. Systemic sclerosis was diagnosed based on ACR/EULAR 2013 criteria.[10] Restrictive lung disease was defined based on the ATS criteria.[11] Based on the spirometry data, all participats were shown to meet the restrictive lung disease criteria. All patients underwent HRCT thorax examination as the gold standard for ILD. Interstitial lung disease was defined based on Wernick's semi-quantitative scoring system category[12] that describes the ILD progressivity by radiological patterns and lung segments involved. Statistical Product and Service Solution (SPSS) version 22 for Windows was used for data analysis. Number and percentage were used for categorical variables. Mean ± standard deviation was used for measurement data following the normal distribution while median was used for non-normal distribution measurement data. This study was approved by the ethical committee of Dr. Hasan Sadikin General Hospital Bandung, Indonesia (reference no: LB.02.01/X.6.5/24/2020). Details on patient selection is shown in Fig 1.

Results

During the study period, 4,653 outpatients visited the Rheumatology Clinic of the hospital, with 1,923 patients with all rheumatology cases, 435 with Rheumatoid Arthritis (RA), 730 with Systemic Lupus Erythematosus (SLE), and 70 with SSc. There were 36 patients in the West Java Systemic Sclerosis Registry from January 2018 to December 2019 who met the criteria for this study. Patient characteristics are shown in Table 1.

More women were affected by SSc in this study (35, 97.2%). The mean age at diagnosis was 40 ± 12 years while the median disease duration was 18 (4-58) months. The most common subtype was dcSSc (n=22, 61.6%), followed by lcSSc (n=14, 38.9%). Raynaud's phenomenon was the most common clinical manifestation found (n=34, 94.4%) followed by sclerodactyly (n=30, 83.3%), skin stiffness (n=23, 63.9%), fingertip scar (n=21, 58.3%),

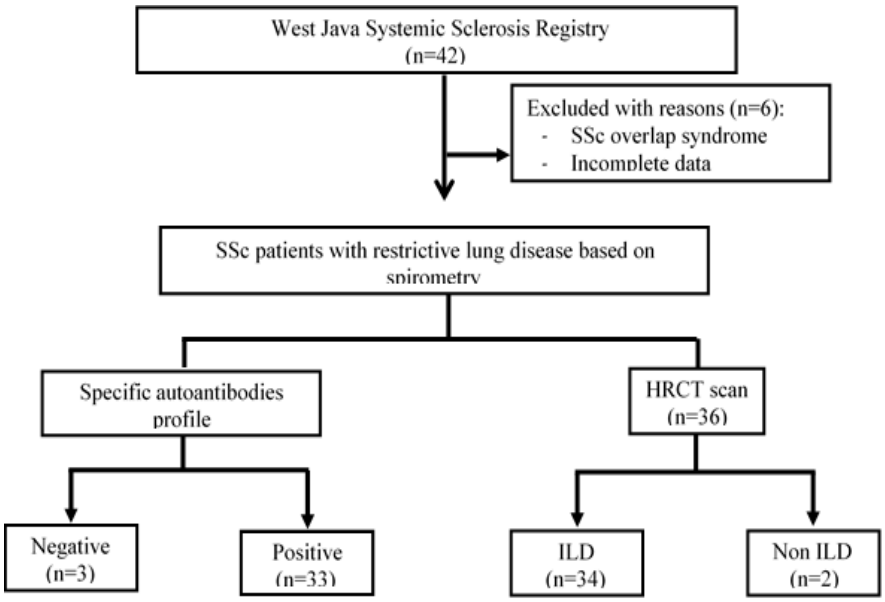

Fig 1. Patient's Selection

Table 1 Patient Baseline Characteristics

Variable	Number of Participants (n=36)
Age at Diagnosis (years)	40 ± 12
Female n (%)	35 (97.2)
Duration of disease (months) median (min-max)	18 (4–58)
Subtype, n (%)	
dcSSc	22 (61.1)
lcSSc	14 (38.9)
mRSS median (min-max)	18 (5-45)
Restrictive Lung Disease, n (%)	
Moderate to Severe	13 (36.1)
Severe	7 (19.5)
Moderate	6 (16.7)
Mild	6 (16.7)
Very Severe	4 (11.0)
Clinical Manifestation, n (%)	
Raynaud's phenomenon	34 (94.4)
Sclerodactyly	30 (83.3)
Skin stiffness	23 (63.9)
Fingertip scar	21 (58.3)
Salt and pepper appearance	20 (55.6)
Telangiectasia	18 (50.0)
Puffy fingers	5 (13.9)
Fingertip ulcer	4 (11.1)
Immunosuppressant therapy	
Methotrexate	32 (88.9)
Cyclophosphamide	4 (11.1)
Mycophenolate sodium	4 (11.1)
Azathioprine	3 (8.3)

dSSc, diffuse systemic sclerosis; HRCT, High Resolution Computed Tomography; lSSc, limited systemic sclerosis; MRSS, modified Rodnan Skin Score; ILD, interstitial lung disease. All participants meet restrictive lung disease criteria. All patients receive immunosuppressant therapy due to SSc severe manifestations

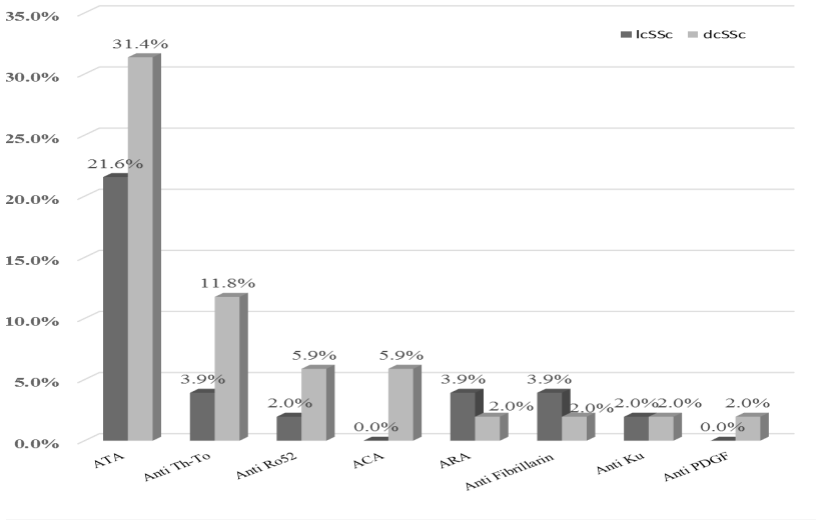

Fig 2. Overview of SSc Specific Autoantibody

salt and pepper appearance (n=20, 55.6%), telangiectasia (n=18, 50%), puffy fingers (n=5, 13.9%), and fingertip ulcer (n=4, 11.1%).

Anti-topoisomerase I was predominantly found in interstitial lung disease, both as a single and an overlap autoantibody, followed by anti-Th/To and anti-Ro52, as depicted in Fig 2. Two types of specific autoantibodies for SSc was positive in half of the patients (50%), followed by one type autoantibody (41.7%), and negative autoantibody (8.3%). The overview of the specific autoantibody is shown in Fig 2.

Almost all patients had ILD (34, 94.4%), with 22 of them had lung fibrosis 22 (61.1%) based on HRCT. Further classification using Wernick's semi-quantitative scoring system category revealedsevere ILD as the most common finding (n=22, 61.1%), followed by moderate cases (n=6, 16.7%), and mild

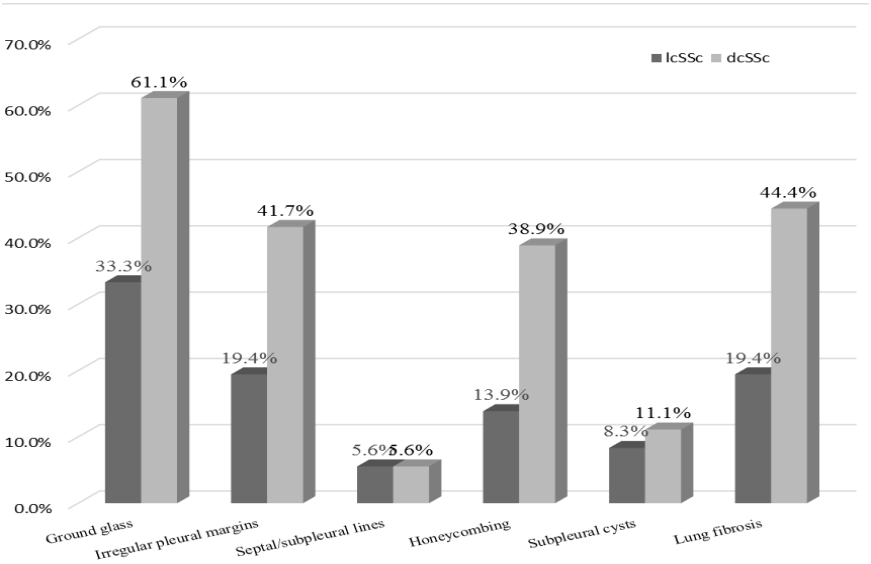

Fig 3. Interstitial Lung Disease Morphology[15]

cases (n=6, 16.7%).[12,13] Severe ILD was found in 41.7% of dcSSc and 19.4% of lcSSc. The HRCT showed usual interstitial pneumonia (UIP) in 52.8% cases, followed by non-specific interstitial pneumonia (NSIP) in 41.7% cases. The ILD morphology is presented in Fig 3.

Discussion

This study describes specific autoantibodies and thorax HRCT in SSc patients. This result differs from other studies and may suggest a difference in characteristics and disease progression of SSc patients in Indonesia. Younger age at diagnosis and shorter duration of illness was found in this study compared to other previous studies in Singapore, Malaysia, Australia, and Europe. [1,2,7,14] This reflects better early case finding in those countries. Interstitial lung disease that occurs in an early stage and younger age is usually severe and have strong relationship with a higher standardized mortality rate.[1,2,15]

Subtype SSc classification is useful for the prediction of organ involvement and disease prognosis. The dsSSc subtype is at a higher risk of death as it is associated with rapid progression and the risk of organ involvement in the early stage of the disease.[16] This study showed that the dsSSc subtype is more common and consistent, with a younger age at diagnosis and shorter duration of illness. Higher prevalence of dcSSc and ATA, when compared to Caucasians, has been reported in Han Chinese, suggesting that racial differences may contribute.[17] The environment is also a trigger factor for SSc, even though it was not fully understood. Some environmental factors that may trigger SSc, include chemical exposure of vinyl, chemical solvents, epoxy resin dan silica. Infection cytomegalovirus (CMV), parvovirus, smoking and alcohol is also play a role in triggering SSc.[16,18]

Specific autoantibodies testing has become the gold standard for the diagnosis of SSc, where it is often associated with the disease progression. An early assessment of specific autoantibodies will give benefits to the patients, especially those suffering from SSc with organs involvement. In this study, we found three dominant specific autoantibodies for SSc, i.e., anti-topoisomerase I, anti-Th/To, and anti-Ro52. Previous studies conducted by Ibrahim *et al*. in Malaysia and Ching *et al*. in Thailand have reported anti-topoisomerase, anti-Th/To, anti-Ro52, and anti-centromere as major autoantibodies in SSc.[14,19] Anti-Th/To and anti-Ro52 are not included in the ACR/EULAR 2013 for SSc diagnosis criteria, due to differences in the populations studied. Specific autoantibodies differences between Asian and European. Singapore, and Malaysia, which are multi-racial countries, have shown differences by population.[2,14] This might be due to the more diversity in human races of the two countries. HLA-DRB1*11 is found in anti-topoisomerase I, while HLA-DRB1*01 and HLA-DRB1*04 HLA-DRB1*05 are found in anti-centromere. In addition, HLA-DRB1*04 and HLA-DQB1*03 are found in Caucasian-Hispanic anti-RNA polymerase.[17,18,20] Studies on chromosomes are quite scarce. Further research is needed in Asian populations, especially in Indonesia, to determine the differences in major autoantibody and disease progression between Asian and European populations.

Specific SSc autoantibodies is associated with organ involvement and an association is observed between autoantibody subtype and clinical manifestation, along with organ involvement.[21,22] In this present study, two-type specific autoantibodies for SSc is positive in half of the participants (50%), followed by one autoantibody (41.7%), and negative autoantibody (8.3%). In several studies using the IIF, immunoprecipitation, and immunodiffusion, only a very small SSc specific autoantibodies are found to have more than one SSc-related antibody.[7,19] Anti-topoisomerase I is the dominant autoantibody found in ILD, both as single and overlap autoantibody, followed by anti-Th/To and anti-Ro52, in which this autoantibody relates to SSc-ILD. This fact is consistent with the finding of this study where anti-topoisomerase was found in 31.4% of diffuse subtype cases and 21.6% of limited subtype cases. Anti-topoisomerase I is often associated with diffuse subtype and interstitial lung disease occurrence. Other studies found anti-topoisomerase I as the most common finding in diffuse SSc with interstitial lung disease.[1,8,10,22] Anti-Th/To is found in 15.7% cases and becomes the second most dominant autoantibodies. These results are not consistent with the literature, where anti-Th/To is found to be less than other autoantibodies. A study in Japan shows only 2-5% of cases with anti-Th/To and none were found in Greece population .[20,21] In this present study, 7 of 8 positive anti-Th/To patients had interstitial lung disease. This result is similar to a previous study in Malaysia and Thailand, which reported SSc with anti-Th/To positively increases the severity and mortality rate due to lung involvement.[14,19] There are differences

in dominan autoantibody found in Japan and Greece population, compared to this study population where Anti-Ro52 was found in 5.9% of diffuse subtype cases and 2% of limited subtype cases. All patients with Anti-Ro52 positive had interstitial lung disease, which is similar to a study conducted by Ibrahim *et al*.[14] Anti-centromere was found in 5.9% of a diffuse subtype with overlapping anti-topoisomerase I autoantibody. This group showed severe interstitial lung disease. This finding is not consistent with the literature, which stated that anti-centromere has a good prognosis because it is often found in a limited subtype with rarely visceral organ involvement.[21] This may be associated with the presence of two or more autoantibodies simultaneously, especially high titer anti-topoisomerase I. Anti-RNA polymerase was found in 3.9% of diffuse subtype cases and 2% of a limited subtype with overlapping anti-topoisomerase I autoantibody. The literature states SSc with anti-RNA polymerase rarely develops into severe interstitial lung disease. This is not consistent with the results. Interstitial lung disease in this group can be associated with the presence of two concurrent autoantibodies, especially high titer anti-topoisomerase I. This study found three dominant autoantibodies, which consist of anti-topoisomerase I, anti-Th/To, and anti-Ro52. Studies conducted by Ibrahim *et al*. in Malaysia and Ching *et al*. in Thailand reported anti-topoisomerase, anti-Th/To, anti-Ro52, and anti-centromere as dominant autoantibodies.[14,19] Anti-Th/To and anti-Ro52 are not included in ACR/EULAR 2013 for the diagnosis of systemic sclerosis. This is because ACR/EULAR 2013 is based on the European and American populations, while there are differences in autoantibody dominant between Asian and European populations.[10,17,23, 24]

This study also discovered three negative cases for specific autoantibodies but with a positive ANA test. Factors that may play a role in autoantibody difference results include autoantibodies test used and autoantibodies titer.[9,22] Euroline immunoblot assay has the ability to detect 13 autoantibodies; however, there are several other autoantibodies that are linked to systemic but not included in this kit, such as anti-endothelial, anti-fibroblast, anti-matrix metalloproteinase, and anti-survivin, thus cannot be examined in this study. A high titer is associated with disease progression.

The HRCT showed that almost all patients had ILD with UIP pattern, which is different from findings of other studies conducted in Europe, in which NSIP is the common pattern.[13,25] UIP is associated with worse prognosis caused by rapid disease progression, presenting a of peripheral septal thickening craniocaudal gradient, bronchiectasis, and honeycombing.[26] Further classification using Wernick's semi quantitative scoring system found that severe ILD was the most common finding, consist with the UIP pattern.[12] ILD progression is related to morphology of lung lesion.[13,25] . This study shows that many patients seek healthcare after later stages of the disease with visceral organ involvement such as interstitial lung disease.

Risk factors for interstitial lung disease in SSc include men, older age, African-American, duration of illness, diffuse subtype, presence of ATA, decreased Forced Vital Capacity (FVC), and biomarkers.[26,27] The participants in this study are mostly women, aged between 30 – 40 years with a duration of illness less than 18 months, diffuse subtype, as well as anti-topoisomerase 1 positive with decreased FVC. Differences in gender, age, race, and duration of illness are observed in this study.

In West Java Province of Indonesia, SSc with ILD is commonly found, with diffuse cutaneous subtype as the predominant group and anti-topoisomerase I, anti-Th/To and anti-Ro52 are the most common autoantibodies observed in the area. In line with the pathogenesis, Anti-topoisomerase I triggers adhesion and activation of monocytes by binding to DNA–topoisomerase I expressed on fibroblasts.[28] This potentially lead to the amplification of the fibrogenetic cascade. Anti-Th/To and anti-Ro52 are also associated with a higher prevalence of SSc-ILD. The HRCT demonstrates predominant SSc with ILD in this population. This study identify similar autoantibodies associated with ILD among SSc patients. However, some limitations should be noted, such as the absence of data on living environment, profession, chemical exposure and previous disease history in the Registry because the registry is still in ongoing.

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Original Article

Clinical Profile of Mucormycosis during the Second Wave of COVID-19 in a Tertiary Care Center in India

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Abstract

Objective: To study the clinical profile and treatment outcome of mucormycosis associated with the second wave of COVID-19 pandemic.

Methods: An observational study was conducted in a tertiary care center over a period of 12 months, including a 6-month post treatment follow up. Study included all COVID positive patients with a clinical and radiological evidence of rhino-orbito-cerebral mucormycosis during the second wave of COVID-19. All patients underwent further diagnostic workups and confirmed cases underwent surgical debridement, and Amphotericin B was started.

Results: A total of 59 patients presented with mucormycosis with the mean age being 52.7 years with unilateral facial and orbital edema as the most common symptoms (28.8%). All were diabetic with HbA1c >7 (54.2%). The mean duration of presentation was 20.7±7.9 days from the onset of COVID-19 infection. Unilateral involvement of the paranasal sinuses was the most common finding in MRI. Early administration of Amphotericin B with prompt surgical debridement was performed in all cases. Orbital exenteration was conducted in nine patients for better fungal load clearance. Patients showed a good response to surgical debridement and prompt medical treatment, with a mortality rate of 27%.

Conclusion: COVID-19 associated mucormycosis is difficult to treat and often presents in late stage. Uncontrolled diabetes, immunocompromised state, and steroid-induced immunosuppression were important risk factors. A close surveillance for early identification and initiation of treatment is mandatory. Repeated surgical debridement to clear the dead tissue is effective to control fungal load.

Keywords: Amphotericin B, Covid-19, invasive fungal sinusitis, mucormycosis

Introduction

Since the emergence of COVID-19 pandemic in March 2020, the medical fraternity all over the world have been constantly witnessing new paradigms of its clinical manifestations and other illnesses associated with this disease. Otolaryngologists have been involved in the management of COVID-19 at various levels and have to face new challenges. One such example was that of the higher than ever number of mucormycosis infections, especially during the second wave of the COVID-19 pandemic.[1,2] An exponential increase in the number of mucormycosis cases was seen in the entire country when compared to the rest of the world.[3] A literature review on mucormycosis would reveal that several studies have been published so far, describing the different aspects of mucormycosis. Here, an attempt is made to revisit the clinical profile as well as the surgical strategies in the

management of mucormycosis under the new scenario of COVID-19 pandemic. The different presentations of these cases, as encountered in a tertiary care center, would be highlighted. The aim is to study the clinical profile of mucormycosis as an effort to throw lights into a few risk factors and to present the details of the medical and surgical management of this disease.

Methods

This study was conducted at the Government Medical College Hospital Kozhikode, which is a tertiary care teaching center in India. An observational study design was followed. The total duration of the study was one year from May 2021 to April 2022, which included inpatient observation as well as a six-month post discharge follow-up of the patient. All patients who were covid positive with clinical and radiological evidence of mucormycosis, as well as a histopathological or microbiological evidence of mucormycosis, were included in the study. A convenience, purposive sampling method was employed and a written informed written consent was obtained from all patients or from their primary care givers (in case of debilitated patients) before enrolment into the study. A scientific committee approval as well as ethics committee approval was obtained from the Institutional Ethics Committee of the Government Medical College Hospital Kozhikode (Ref No. GMC KKD /RP 2022/ IEC/14).

After the initial work-up, all patients had a CT scan and MRI of the nose and paranasal sinuses. All patients had a diagnostic nasal endoscopy done and samples were taken for KOH smear preparation, fungal culture, and histopathology examinations. The confirmed cases of mucormycosis were taken up for surgical debridement and Amphotericin B, which was already started empirically, would be continued.

Results

A total of 59 cases were enrolled in the study, mostly referred from peripheral hospitals. Most of the patients were males (67.8%), and the majority of patients belonged to the age group of 45-60 years. All of them were detected to be COVID-19 positive either during the admission or were previously infected within the past 2 months. About 59.3% had received the first dose of vaccination before the infection and one had received two doses of vaccination. Almost all the patients were diabetics, where 45.8 % had suffered from the disease for more than 10 years while six were recently detected as diabetics and were not on any medications. One patient with type 1 diabetes had been on insulin for the last 30 years. Hypertension was the most common coexisting disease (18.6%), followed by chronic kidney disease (16.9%) and coronary artery disease (8.5%). One patient had undergone renal transplant 10 years back for diabetic kidney disease. Most patients presented within an average

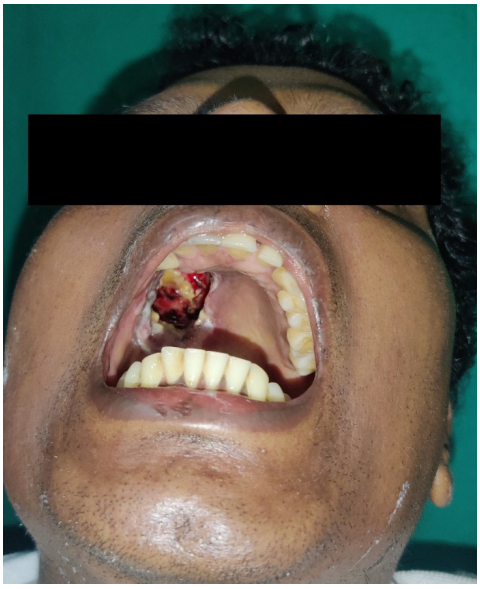

Fig 1. Palatal Perforation

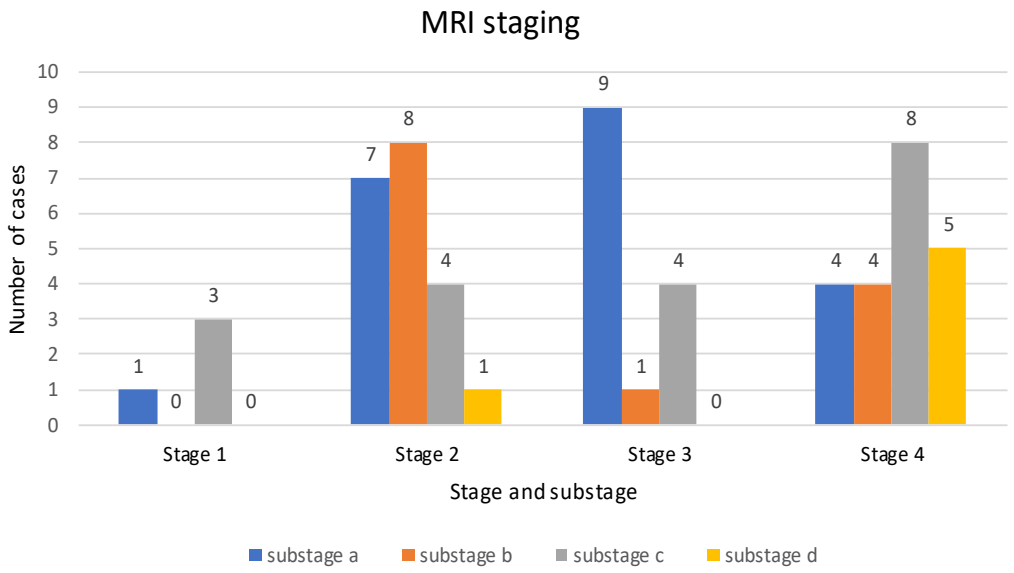

Fig 2. MRI staging based on the Honavar staging for COVID-19 associated rhino-orbital-cerebral mucormycosis cerebral mucormycosis [1]

duration of 20.78±7.9 days from the day of onset of covid symptoms. Eleven patients were detected to experience an active covid infection at presentation, with symptoms of mucormycosis. About 48% patients had received intravenous corticosteroids as a part of the covid treatment.

Unilateral orbital and facial edema (28.8%) were the most common presenting symptom, followed by headache in 25.4% patients. In the majority of patients, the disease was lateralized to the right side; however, six patients experienced a bilateral involvement. The clinical examination revealed periorbital and facial edema as the primary signs in 44.1% patients, while nasal discharge was seen in 22 patients, of which 15 had a characteristic blackish discharge. Around 22% of these patients experienced a numbness of the face. A defective vision was noted in 20 patients of which 17 had only perception of light in the affected eye. Ptosis and ophthalmoplegia were noticed in 28.8% cases while proptosis was seen in 21 patients. There were 3 patients with a lower motor neuron type of facial nerve palsy and one patient presented with palatal perforation **(Fig 1).**

Blood investigations showed high levels of glycated hemoglobin with the HbA1c level of >7 in 54.2% of the patients. The ESR and C-reactive protein (CRP) levels were elevated in all patients. In all cases referred to this center, computerized tomography (CT) scan of the nose and paranasal sinuses was the first radiological investigation done. An MRI scan with a gadolinium contrast was done in all, except in the 10 patients with chronic kidney disease. Based on these findings the patients were staged as per the Honavar staging for COVID-19 associated rhino-orbital-cerebral mucormycosis.[4] Of the 35.5% patients who presented with a STAGE 4 disease and five (5) patients were in STAGE 4d, 8 in stage 4c, four (4) in STAGE 4a and 4b, respectively. The remaining 23.7% patients had a STAGE 3 and 33.8% had STAGE 2 disease (**Fig 2).**

Unilateral involvement of the paranasal sinuses was the most common finding noted. Most commonly involved sinuses were the ethmoids (89.8%), followed by the maxillary sinus (79.7%). Despite being a rare finding, isolated sphenoid sinus involvement was observed in one patient. Orbit involvement was seen in 57.6 %, of which 22% were an early involvement of the medial part of orbit as a result of the erosion of lamina papyracea. An involvement of the pterygopalatine fossa was noted in 31 patients (52.5%). Intracranial extension was seen in 19 patients, mostly in the form of cavernous sinus involvement mainly

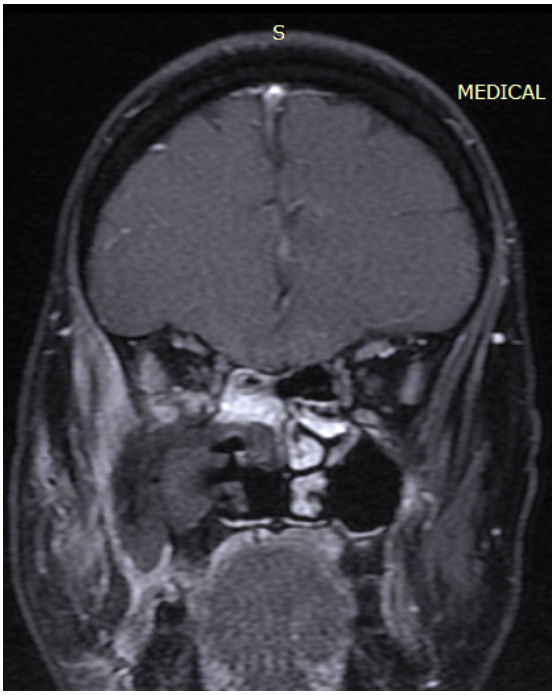

Fig. 3 Black Turbinate Sign

through the superior orbital fissure (78.9%). There were four cases of direct extension into the frontal lobe through the cribriform plate. Diffuse CNS involvement were seen in five (5) patients in the form of multiple infarcts, possibly embolic dissemination of the fungal materials. A diagnostic nasal endoscopy was done in all patients and 39 patients, showing the characteristically described black eschar in the nasal cavity. A discoloration of middle turbinate was noticed as the earliest finding in the study group. A nasal swab and a nasal mucosal biopsy were done. KOH mount gave a positive report in 69.5 % of patients. Fungal culture yielded Rhizopus in 64.4% of patients. Both KOH mount and fungal culture were found positive in 38.9 %. Histopathological examination and special staining were done in all patients and invasive fungal hyphae were demonstrated in 62.5% of patients. All the specimens were sent for microbiological and histopathological confirmation after surgical debridement was performed. In 14 cases, a coexisting invasive mucormycosis and aspergillus infections were present.

Patients were treated empirically using intravenous Amphotericin B based on clinical suspicion. Most patients underwent their first surgical debridement within a week of admission and were later followed up with daily clinical progress with the ESR and CRP measurement repeated once every three days. In patients who did not show a significant improvement, further MRIs were performed to assess the need to undergo subsequent endoscopic examinations and debridement. Most common focus of residual disease during the re-examination was found to be at the pterygopalatine fossa. A repeat debridement had to be done in 86.4% of cases and 21 patients (35.5%) even had it done three times. Retrobulbar amphotericin injection for local control were given in four (4) patients, who had significant orbital involvement. Despite all these measures, orbital exenteration had to be carried out in nine patients for better clearance of fungal load. One patient experienced a frontal cerebritis which evolved into an abscess, which was drained by the neurosurgical team. Two patients underwent a high-resolution CT scan of the thorax in view of their persistent chest symptoms and were later found to have pulmonary involvement too.

The cumulative dose of the amphotericin B injection for most patients was 3gm (56 %), whereas 4 gm had to be given to 12 patients (20.3%) with stage 4 disease. The mean length of hospital stay was 27.7 days. A consolidation therapy with oral Posaconazole was started for patients on discharge. Among the patients in the study group, 13 (22%) succumbed to the disease during the hospital stay. Two patients had to be readmitted during the period of one month after the discharge but expired soon afterwards. One patient died as a result of his medical ailments within two months after the discharge. The remaining 43 patients showed a good clinical improvement during the follow-up period and were relatively symptom free at the end of the follow-up period.

Discussion

Mucormycosis is an uncommon and aggresive fungal infection that usually affects patients with an altered immunological system.[5] This disease was first described by Fürbinger in Germany (1876). Arnold Paltauf published the first case of disseminated mucormycosis in 1885.[5] The prevalence of mucormycosis in India is estimated to be 0.14 cases per 1000 population, or approximately 80 times of the worldwide estimated rate.[5] Among the different types of mucormycosis, the Rhino-orbito-cerebral form is considered the most common type, especially in patients with uncontrolled diabetes mellitus.[6] Explanations

have been provided to explain the increased incidence of mucormycosis cases in diabetic patients. The most accepted theory is that persistent hyperglycemia causes an impaired chemotaxis affecting the phagocytic function of neutrophils. In addition, the ketoacidosis impairs the binding of iron to transferrin, thus increasing the amount of free iron in the body that in turn promotes fungal growth.[7]

A steroid-induced immunosuppression, as well as immune dysregulation associated with COVID-19 infection due to reduced numbers of CD8 and CD4 T lymphocytes, have also been considered as important factors in the current setting.[8] A recent Indian study by Honavar *et al* reported a mean age of 51.9 years and a male preponderance of 71%.[4] In a meta-analysis of 600 articles conducted by Jeong *et al* with 851 cases, the median age was 51 years and 63% patients were males.[9] This study yielded a similar finding with the mean age being 52.7 years and a significant male predominance of 67.8%. In their meta-analysis, Jeong *et al* found diabetes mellitus as the most common underlying condition.[9] They found the use of corticosteroids at the time of presentation as the most common predisposing factor (33%). All patients in this study were diabetics of which six of them were recently detected diabetics and almost 48% patients had received intravenous corticosteroids as a part of the covid treatment. The patients presented with features of mucormycosis on an average of 21 days from the day of onset of COVID infection. On the contrary, in a review of cases from 18 countries, Hoenigl *et al* reports a median duration of 10 days between COVID-19 and mucormycosis diagnosis.[10] In patients without a concurrent covid infection, uncontrolled diabetes with ketoacidosis played a significant role. In this present study, headache and facial edema remain the earliest the presenting complaints, followed by proptosis, ptosis and visual disturbances. A complete loss of vison was seen in 4 patients (6.7%) due to a central retinal artery occlusion. Central retinal artery occlusion bears an incidence of 16-20% and is thought to be due to the vasculitis caused by the direct infiltration of the fungi in to the retinal artery.[11] Facial paresthesia was also commonly seen in patients with extensive orbital involvement. A black necrotic eschar seen on the palate or nasal mucosa were noted in 39 patients. MRI with gadolinium contrast assists in staging the disease and planning the extent of surgical debridement. The classical black turbinate sign described by Safder *et al* in MRI,[12] occurs due to the occlusion of small vessels causing a lack of contrast enhancement of invaded mucosa **(Fig 3).**

Yield from a combination of diagnostic nasal endoscopy, microscopy and fungal culture were usually high. This warrants a close follow up of COVID-19 recovered patients with routine screening nasal endoscopies and guided swabs for early detection.

Treatments for mucormycosis of paranasal sinuses revolves around a combination of systemic antifungal therapy, the reversal of immunocompromised state and radical surgical debridement. A proper and rapid correction of the metabolic abnormalities along with the complete removal of all infected tissues gives a definite benefit.[13] Time is a crucial factor as far as the treatment is concerned. Any inadvertent delay can significantly affect the treatment outcome. Treatment with liposomal or conventional Amphotericin B should be given as per recommendations, along with strict glycemic control and continuous renal function monitoring. There was a difficulty in procuring liposomal Amphotericin B for these patients, as there was a nation-wide shortage of Amphotericin B owing to the rapid increase in the number of mucormycosis cases. But somehow, the patients were put on a combination of liposomal or conventional Amphotericin B, depending on the availability. Surgical debridement needs to be done at the earliest. Surgical debridement slows the progression of the disease, reduces fungal load and it provides a specimen for culture. Several factors can delay the early surgical intervention. Even though the earliest surgical intervention was on the second day of admission, in few other cases the intervention had to be delayed due to an active covid infection in the patient. Even though the recommendations at that period was to defer the surgical debridement in COVID positive patients, an early intervention and emergency surgical debridement had to be done, to save few critically ill patients.

For the post procedure clinical assessment, patients had their routine ESR and CRP value estimation, which were helpful in marking the progress of the disease. A repeat MRI with contrast was taken on noticing any clinical deterioration or increasing levels of the inflammatory markers. The subsequent scan helped in picking up any residual disease in the orbit and pterygopalatine fossa. These areas are usually neglected during the initial surgical debridement because on visualizing an intact posterior wall of maxilla and lamina, the surgeon hopes that the disease would

not have breached the intact bony wall. Even though Plowes *et al* mentions 100% involvement of the pterygomaxillary fissure in their series of five patients, the present study found the area to be involved in 52.5% (31 patients).[14]

In these cases, complete clearance of fungal load is mandatory; hence, a good exposure of the surgical field is essential. For this reason, a modified endoscopic Denker's method, as well as maxillary posterior wall removal, devitalized tissue debridement in the pterygopalatine fossa, and pterygoid base drilling would be a good option to ensure a reasonably good clearance. Even though there is no clear-cut evidence, topical antifungal therapy is primarily used adjunctively in invasive fungal sinusitis.[15] Considering the relatively low systemic absorption, topical amphotericin gel for local application was used in some cases, at surgical sites where the complete clearance was still doubtful. While managing the orbital counterpart of this disease, cosmetic outcome becomes an important aspect and hence a trial with retrobulbar amphotericin injections should always be considered first. In this series, a retrobulbar amphotericin injection was given in 4 patients. A meticulous orbital debridement helps in removing all devitalized orbital tissues and gives an option of preserving the globe. It helps in eliminating the fungal load and can very well prevent a dreaded intracranial extension. But in a worsening clinical scenario, with cavernous sinus thrombosis, an orbital exenteration is warranted so as to avoid an intracranial involvement. Plowes *et al.* in their study has considered orbital apex syndrome and cavernous sinus thrombosis as the only criterion for an orbital exentration.[14] Seiff *et al* considered removing the infected orbital tissues conservatively and irrigating the remaining tissue with a solution of Amphotericin B.[16] They claimed that their technique avoided an orbital exenteration in most cases. Despite doing a thorough orbital debridement, using local Amphotericin B wash and retrobulbar injections in selected cases, an orbital exenteration had to be done in 9 cases.

In a study by Jeong *et al*,[9] the overall mortality was 46% whereas in the review by Hoenigl *et al* [10] mortality was reported in 37% cases. In this series, the mortality was only 27 % which was possible due to the prompt response of the surgical team in clearing the fungal load along with maximal medical therapy. Hence a close surveillance post covid, early recognition of symptoms and early initiation of treatment can drastically improve the prognosis in patients. In conclusion, mucormycosis cases spiked up in India in association with COVID 19 infection and added to the disease morbidity and mortality. Uncontrolled diabetes, immunocompromised state and steroid-induced immunosuppression were the important risk factors for COVID-19 associated mucormycosis. Routine nasal endoscopies, meticulous sampling and an MRI with gadolinium contrast in suspicious cases will help in accurate diagnosis. A thorough monitoring of immunocompromised state and an early initiation of liposomal amphotericin B therapy is indicated, along with repeated surgical debridement till a complete clearance of dead tissue is obtained. These measures can help in reducing the mortality associated with this disease to a great extent.

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Original Article

Oxidative Stress in Seminal Plasma Negatively Influences Sperm Quality in Infertile Males

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Abstract

Objective: To investigate the association between the malondialdehyde concentration in the seminal plasma of infertile men and sperm quality.

Methods: This case-control study included 60 male participants, ranging from 25-40 years old, with half of them were fertile and the other half were infertile. Semen analysis was performed as per the WHO standards, and spectrophotometric measurement of the seminal plasma malondialdehyde level was done.

Results: Results showed that infertile men had significantly a higher mean level of malondialdehyde in their seminal plasma than fertile men ($p<0.001$), which was inversely associated with sperm count and motility. Also, malondialdehyde was positively associated with abnormal sperm morphology.

Conclusions: Elevated malondialdehyde levels in the seminal plasma are associated with poor sperm quality. Malondialdehyde testing can, therefore, be used to diagnose and predict the outcome of male infertility. Antioxidants should also be administered to men with infertility to help counteract the effects of oxidative stress.

Keywords: Male infertility, malondialdehyde, sperm quality

Introduction

It has been estimated that fifteen percent of couples experience infertility due to various causes. Infertility is defined as the failure to achieve a spontaneous pregnancy after twelve (12) months or more of frequent, unprotected sexual intercourse.[1] Nearly half of all infertility cases can be traced back to male factors, which are just as important as the female factors.[2] The global prevalence of infertility ranges from 2.5% to 15%, which corresponds to at least 30 million infertile males.[3]

Numerous studies have pointed out that oxidative stress, which is a condition marked by an imbalance between the generations of reactive oxygen species (ROS) and antioxidant defense mechanisms, as a newly discovered cause of unexplained male infertility.[4,5] When kept within healthy limits, the ROS mediate vital physiological activities crucial to ensuring normal male reproductive functions, including sperm viability, maturation, hyperactivation, sperm capacitation, sperm motility, acrosome reaction, and oocyte interaction.[6,7] However, excessive amount of ROS can lead to infertility via various mechanisms, i.e., DNA damage, lipid peroxidation, enzyme inactivation, and protein oxidation in spermatozoa.[7] Spermatozoa are extremely vulnerable to oxidation because of a high concentration of unsaturated fatty acids found in their membranes and the absence of cytoplasmic antioxidant enzymes. As a result, oxidation has a negative impact on the quality and functionality of the sperm.[7,8] Thus, this study aimed to evaluate MDA as a marker of oxidative stress and investigate its relationship with the quality of sperm in infertile males.

Methods

The present case-control study was conducted at the Department of Biochemistry and In-vitro Fertilization (IVF) center of the MGM Medical College and Hospital, Aurangabad. This study was carried out from January 2013 through December 2013 after getting the approval from the Institutional Ethical and Research Committee (Reference No: EC/056/2012, dated 02 November 2012). The case group consisted of 30 infertile men with abnormal semen analysis between the ages of 25 and 40 years, whose wives had not conceived after a year of having regular, unprotected sex. Thirty healthy, fertile male volunteers in the same age range who were in good health and had normal semen parameters were used as controls.

Patients in the case group were excluded from the study if they had testicular damage, varicocele, leukocytospermia, hypogonadism, cryptorchidism, heart disease, tuberculosis, genital tract infections, renal disease, diabetes mellitus, or prolonged illness. Before starting the study, a written consent was obtained from both infertile males and healthy controls.

Semen samples for this study were collected from subjects and controls after a period of abstinence of three to four day . The specimens were obtained by masturbating into wide-mouth sterile plastic containers and analyzed within an hour of collection. After letting the semen liquefy for at least 30 minutes, it was analyzed to measure sperm concentration, motility, and morphology as described in the WHO guidelines.

MDA levels were analyzed by the method described by Nourooz-Zadeh *et al*.[9] In brief, the first semen sample was centrifuged after liquefaction to get the seminal plasma. Then, 100 µl of seminal plasma, 1,000 µl of 0.67% TBA (thiobarbituric acid), and 500 µl of 20% TCA (trichloroacetic acid) were added and incubated for 20 minutes at a temperature of 100^0C. After centrifugation at 12,000 rpm for 5 minutes, the optical density (OD) of the supernatant was taken at 532 nm using spectrophotometry. The MDA concentration was calculated using the molar extinction coefficient for the MDA-TBA complex of 1.56×10^5 $mol^{-1}Lcm^{-1}$.

Data collected were statistically analyzed using the IBM SPSS statistics version 20. Data were presented as mean±SD. The statistical differences between cases and controls were established by student-independent sample t-test. To understand how the variables related to one another, a Pearson correlation analysis was done. Values were considered statistically significant when $p<0.05$.

Results

Results of sperm characteristics and seminal plasma MDA comparison between infertile and fertile males are listed in Table 1. The level of MDA in the seminal plasma of infertile men was significantly higher than that of the fertile control group ($p<0.001$). Subjects with infertility had significantly lower sperm counts and motility compared to those with fertile men and the abnormal sperm morphology was demonstrated to be significantly higher in infertile males than in normal, healthy fertile males ($p<0.001$). MDA was significantly negatively correlated with sperm count and motility in infertile males and are presented in Fig 1 and 2, respectively. However, there was an insignificant positive correlation of MDA with sperm abnormal morphology in infertile males, which is presented in Fig. 3.

Discussion

Through the modification of the membrane fluidity and permeability, as well as reducing the sperm functional competence, oxidative stress can negatively impacts sperm functions. To be able to evaluate the membrane damage,

Table 1 Comparison of Sperm Characteristics and MDA between Fertile and Infertile Males

Parameter	Fertile male (n=30)	Infertile male (n=30)
Sperm count (millions/mL)	79.17±10.97	32.03±11.29*
Sperm motility (%)	74.57±5.67	32.66±8.18*
Sperm abnormal morphology (%)	17.93±3.77	35.4±4.90*
MDA	1.56±0.34	3.00±0.49*

*Highly significant ($p<0.001$); MDA: Malondialdehyde

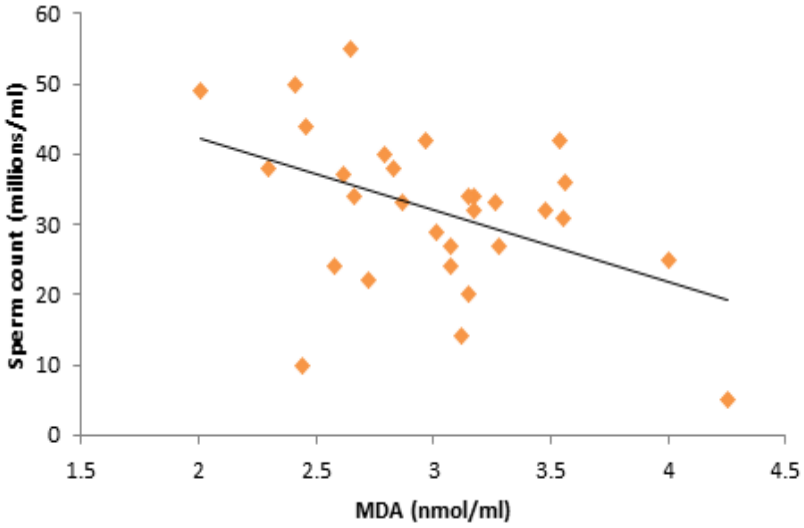

Fig. 1 Correlation between MDA and Sperm Count in Infertile Males (r=-0.447; p<0.05)

the quantity of MDA, the final product of lipid peroxidation, can be taken into account.[10]

In the present study, a significant increased level of MDA was observed in the seminal plasma of infertile males as compared to the fertile group. This is in accordance with the studies done by Atig *et al.*[11], More *et al.*[12], and Dorostghoal *et al.*[13] who also reported increased seminal MDA in infertile male patients. Similarly, a study conducted by Muley and Muley[14] has also demonstrated insignificant higher seminal plasma MDA level in asthenoteratozoospermic and azoospermic males as compared to normozoospermics.

However, a significant rise in MDA levels was observed in the oligoasthenoteratozoospermic

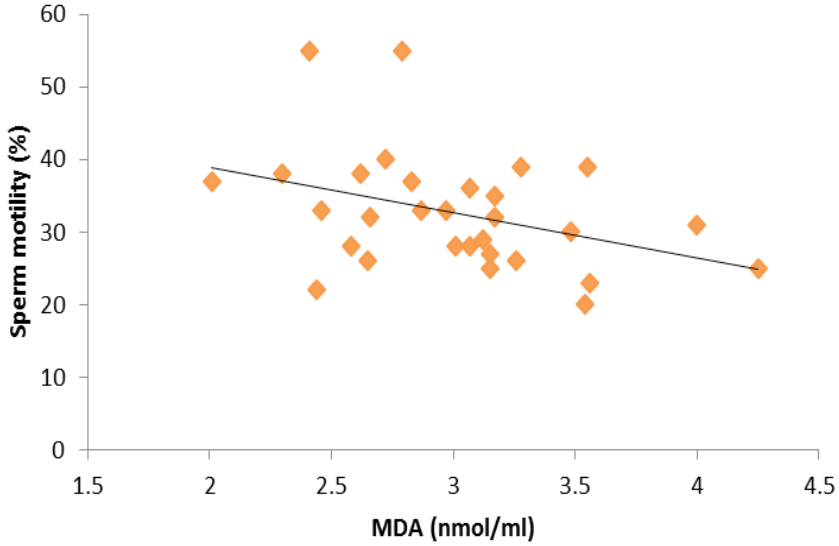

Fig. 2 Correlation between MDA and Sperm Motility in Infertile Males (r=-0.374; p<0.05)

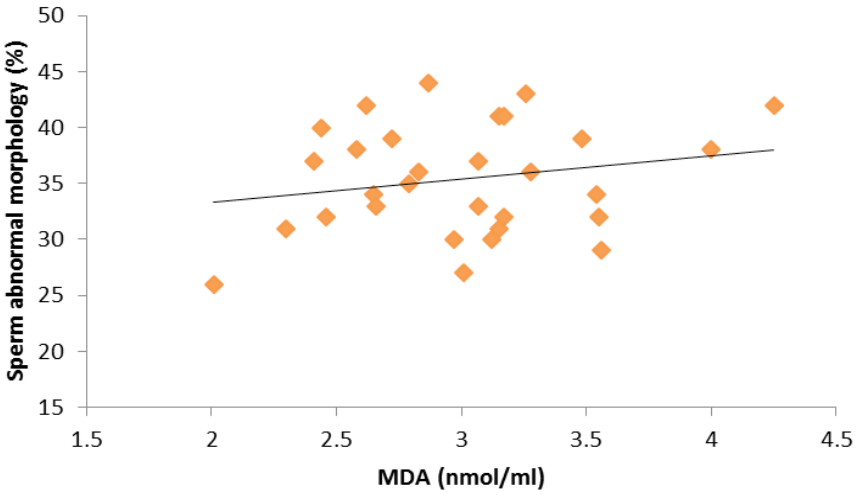

Fig. 3 Correlation between MDA and Sperm Abnormal Morphology in Infertile Males (r = 0.208; p>0.05)

group. In contrast, Palani *et al*.[15] did not find any significant differences in the MDA levels between fertile and infertile males in their study. The disparity between the results of the aforementioned research can be attributed, in parts, to the variability of those studies with regard to patient selection, methodology, and oxidative stress assessment techniques, as well as the genetic and racial characteristics.[15] Increased MDA levels in the seminal plasma of infertile males indicating excessive ROS is responsible for the lipid peroxidation of the membrane lipids. Because the sperm plasma membrane contains a high concentration of polyunsaturated fatty acids (PUFAs), which are essential for ion transport and membrane fluidity, the peroxidation of the PUFAs in the membrane by excessive ROS disrupts the functions of sperm membrane, thus decreasing the ability of spermatozoa to fertilize.[12] It has been demonstrated that both qualitative and quantitative sperm abnormalities exist in the semen of infertile males. The sperm count and motility significantly decreases among infertile men when compared to the fertile ones. Also, the abnormal morphology of sperm has been reported to be significantly high among infertile males when compared to normal healthy fertile males. The findings of this present study indicate that the elevated oxidative stress in the seminal plasma of infertile males is associated with poor sperm quality. These results are well supported by the findings of previous studies.[12,14]

The MDA has been demonstrated to have a significant negative correlation with sperm count and motility in this study. A positive correlation was also observed between seminal MDA and sperm abnormal morphology. These results are in line with the findings of More *et al*[12] and Mehrotra *et al*.[16] Dorostghoal *et al*.[13] also reported the presence of significant negative correlations between MDA levels and sperm motility and normal morphology. An excessive amount of ROS can decrease sperm motility, most likely through a rapid loss of intracellular ATP leading to axonemal damage, decrease sperm viability, and increase mid-piece morphological defects with deleterious effects on sperm capacitation and acrosome reaction.[17,18] According to the findings of this study, the ROS are responsible for inducing base modification, DNA strand breakage, and chromatin cross-linking, all of which are detrimental to the integrity of the DNA in the sperm nucleus.[19] On the other hand, DNA damage caused by high levels of ROS may hasten the process of germ cell apoptosis, resulting in a drop in sperm counts associated with male infertility.[20] Due to the small sample size and lack of follow-up of patients in the present study, among other limitations, large-scale prospective investigations are needed to strengthen the findings. In conclusion, it is indicated that increased oxidative stress, as reflected by increased MDA levels in seminal plasma, is associated with poor sperm quality, as reflected by decreased sperm count,

decreased sperm motility, and also increased abnormal sperm morphology as observed in infertile males. Therefore, determination of the MDA levels in seminal plasma can help the diagnosis and prognosis of male infertility. To verify these results, nevertheless, additional research with a large sample size is required.

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Original Article

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Effect of Eye Exercises on Computer Vision Syndrome among Medical Students in A University in Indonesia

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Abstract

Objective: To determine the effect of eye exercises on Computer Vision Syndrome among batch 2019 medical students of the Faculty of Medicine, Universitas Sumatera Utara, Indonesia.

Methods: This study used analytical true experimental with a Pretest-Posttest Control Group Design. Sample consisted of 86 respondents divided into two groups, control and experimental (intervention) groups, was used. Each group consisted of 43 respondents who were sampled randomly using the simple random sampling technique. Data were collected using the Computer Vision Syndrome Questionnaire (CVS-Q) and analyzed statistically with a p-value of <0.05 considered significant.

Results: A decrease in the score of Computer Vision Syndrome in the experimental (intervention) group after the eye exercise was observed with a p-value of 0.001 (<0.05).

Conclusion: Eye exercise affects the Computer Vision Syndrome.

Keywords: Computer vision syndrome, eye exercise

Introduction

Computer Vision Syndrome (CVS) is defined as a complex eye discomfort condition in the form of visual impairment symptoms caused by prolonged exposure to digital displays.[1] Globally, it is estimated that around 60 million people experience this syndrome, with a million new cases identified each year.[2] The pathophysiology of this syndrome is divided into three mechanisms: ocular mechanism of dryness and redness of the eyes with a reduction in blink rate; visual mechanism that leads to blured vision, double vision and slow focus change; and extraocular mechanism associated with non-ergonomic posture in front of the computer screen that causes musculoskeletal symptoms such as headaches and shoulder pain. [3] The COVID-19 pandemic poses a risk of increasing cases of Computer Vision Syndrome due to changes in the environment for study because of the implementation of the online learning policy.[4] Garg *et al.* has reported that more than 70% of medical students of Rama University, India, spent more than 4 hours on their computers. About 40% of students know about CVS, but only 10% take CVS precautions.[5] Another study by Muma *et al*. on 348 students in Kenya, shows a prevalence of CVS of 60.4%, with a low level of knowledge about CVS (46.8%) and 40% of these students did not take preventive measures against CVS. This shows that student's prevention efforts against CVS are still relatively low.

Eye exercises can be considered as a non-pharmacological therapy to prevent and reduce symptoms of Computer Vision Syndrome. Eye exercises therapy is a series of movement performed repeatedly by the eyes to train our eye muscles and its surroundings to be elastic and strong, relax the eyes as to reduce discomfort in the eyes.[6]

A study by Intan Putri *et al*. on eye exercise effectiveness in CVS among nursing students of Riau University concluded that there was a significant difference in the decrease of CVS scores in the experimental group after

the eye exercise intervention.[7] Thus, the purpose of this study was to determine the effect of eye exercise on reducing and preventing CVS complaints among medical students of Universitas Sumatera Utara who were involved in online learning during the COVID-19 pandemic. Different types of eye exercise selected by the researcher with a consideration of feasibility of effective implementation from the time and place perspective were included. The eye exercises were carried out twice a week for one month, with a duration of approximately 10 minutes and can be done anywhere without using any tool.

Methods

This was a quantitative study with a true experimental pre-and post-test design. In this design, two group were randomly selected, Samples was divided into two groups (control and intervention). The intervention group was given eye exercises, while the control group was not given an intervention. Each group was asked to fill CVS-Q one day before doing eye exercises and one day after the last day of doing eye exercises. This study was performed from September to October 2022. Participants were selected using random sampling technique, resulting in 86 medical students of Universitas Sumatera Utara meeting the inclusion and exclusion criteria. The inclusion criteria applied were medical students with the habit of using computers at least 4 hours a day and willing to participate in the study. Participants were excluded if they did not agree to participate. The Computer Vision Syndrome score data were obtained by using the CVS-Q (Computer Vision Syndrome-Questionnaire) from Segui *et al*. which was distributed before and after eye exercises. Ethical approval for this study was granted by the Health Research Ethical Committee, Faculty of Medicine, Universitas Sumatera Utara under the ethical clearance number 797/KEPK/USU/2022.

Eye exercises in this study were carried out twice a week for one month, with a duration of exercise of approximately 10 minutes. Each eye exercise session involved the following sequential steps: blinking, palming, figure of eight, eye movement, 20-20-20 rules, and near and far focus.

In details, the instructions given for the eye exercise were as follows: (1) Sit on a chair with a straight posture and straight head position as comfortable as possible; (2) Blink 1-2 times every 10 seconds; (3) Rub palms together to warm the eyes, put them on the eyes and breathe deeply for 1 minute; (4) Then focus on an area on the floor around 8 feet away, and move the eyes in the shape of a figure 8. Trace the imaginary figure of 8 for 30 seconds, then switch direction; (5) Move the eyeball right and left, repeat 3 times; (6) Move the eyeball up and down, repeat 3 times; (7) Move the eyeball in a clockwise circle and counterclockwise, repeat 3 times; (8) Direct the eyes to look at an object 20 feet away for 20 seconds, every 20 minutes; (9) Lastly, position the fingers a few inches from the eyes, then focus the gaze on the fingers. Move the fingers away and slowly point them back closer to eyes.

The CVS score were then analyzed using the Wilcoxon test, with a p-value of <0.05 considered significant. Age and gender were used as the secondary data for individual characteristics of the subject.

Results

Data on subject characteristics were obtained from the 86 respondents in terms of gender and age as seen in Table 1. Most respondents were women and 21 years old, which was probably due to the fact that most students in that age were working on their mini-thesis, increasing their computer use.

Table 2 and Fig. 1 demonstrate that there are three symptoms that were frequently experienced before and after intervention, i.e., headache, tearing, and itching.

As described in Table 3, the intervention group experienced a significant decrease in the CVS score after the eye exercise, with a p-value of 0.001 while the control group did not show a decrease in the CVS score (p-value=0.802). Two respondents in the intervention group showed increased CVS scores after intervention, while 17 respondents experienced an increase in the CVS score in the control group.

Table 1 Subject Characteristics

Variable	n=86
Age (years)	
20	27 (31.4)
21	53 (61.6)
22	6 (7)
Sex	
Female	66 (76.7)
Male	20 (23.3)

Table 2 Computer Vision Syndrome Symptoms Before and After Intervention

Symptom	Before intervention	After intervention
	n=86	n=86
Burning	25 (29)	20 (23.2)
Itching	74 (86.1)	61 (70.9)
Feeling of a foreign body	67 (77.9)	43 (50)
Tearing	75 (87.2)	64 (74.4)
Excessive blinking	34 (39.6)	29 (33.7)
Eye redness	57 (66.3)	43 (50)
Eye pain	60 (69.8)	48 (55.9)
Heavy eyelids	47 (54.6)	40 (46.6)
Dryness	54 (62.8)	52 (60.5)
Blurred vision	60 (69.8)	48 (55.9)
Double vision	27 (31.4)	21 (24.4)
Difficulty focusing for near vision	32 (37.2)	19 (22.1)
Increase sensitivity to light	47 (54.7)	36 (41.9)
Colored halos around objects	21 (24.5)	14 (16.3)
Feeling that sight is worsening	43 (50)	32 (37.2)
Headache	77 (89.5)	67 (77.9)

Table 3 Wilcoxon Test Analysis Results of Control and Intervention Groups

Ranks	Group	
	Control	Experimental
	(n=43)	(n=43)
Negative ranks[a]	17	2
Positive ranks[b]	25	37
Ties[c]	1	4
p Value	0.802	0.001

Note: * a. (score (pre) < (score (post); b. (score (pre)> (score (post); c. (score (pre)= (score (post)

Discussion

Computer and VDT(Visual Display Terminal) have become an essential part of modern lifestyle; thus the term of "Computer Vision Syndrome" is then coined to describe a group of symptoms experienced after a prolonged use of VDT. Therefore, by doing eye exercises could help to prevent and reduce the occurrence of Computer Vision Syndrome

Before the intervention, symptoms that were mostly mentioned by the participants were headache, tearing, and itching. However, after the intervention, the number and the percentage of the symptoms declined. This

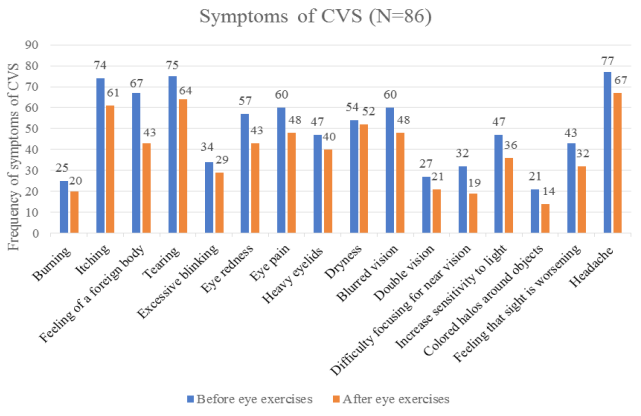

Fig 1. Symptoms of CVS

result supported the study by Ranasinghe and Altalhi that stated headache as the most common symptom mentioned.[8,9,10] Excessive tear production is not directly linked to the duration of VDT use, but to an increased reflex in the eye to increase tear production [11].

Analysis using the Wilcoxon test in the intervention group produced a p-value of 0.001 ($p<0.05$), showing that the eye exercises affect the reduction of the CVS score in this group. This is in line with the findings of previous studies stating a significant decrease in CVS score after eye exercises[7,12]. A significant reduction of CVS in this study may also be linked to the fact that the respondents complied to do the eye exercise properly and routinely twice a week for a month.

Eye exercises are proven to improve the vision and performance of the eyes muscular and motor activities. Palming helps to relax the ocular muscles and all sensory nerves related to vision. Eye movements and figure of eight help the eyes muscle to control the back and movement of the eye's lens, to achieve sights at multiple distances[13]. Blinking replenishes the tear film by redistributing tears from the lacrimal glands and lipids from the meibomian glands on the surface of eye[14]. Thus, eye exercises are shown to positively affect the Computer Vision Syndrome. However, this study also has limitations, such as the small sample size and short period of eye exercises, leading to less data variation. Therefore, a future study should have more respondents and extended period of eye exercises. Other types of eye exercises may also be applied in order to get more representative results.

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Original Article

Sedative Effects of Intraperitoneal Diazepam in Mice

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Abstract

Objective: To determine the effectiveness of Diazepam in comparison with Phenobarbital.

Methods: Twenty-seven male Swiss Webster mice were used and randomly divided into three groups of negative control (NS), positive control (phenobarbital), and diazepam group. Two tests were performed on these groups: Traction Test and Fireplace Test. Pupillary diameter was also observed.

Results: A significant difference based on the Kruskal - Wallis statistical test was observed between the positive control and the diazepam group (<0.05) in the traction test, which was also true for the fireplace test ($p<0.05$). The pupillary diameter in the test animals in the positive control and diazepam group was not statistically significant ($p>0.05$).

Conclusion: Diazepam has a better sedative effect than Phenobarbital. The sedative effect produced by Diazepam is stronger, with faster onset and longer half-life than the Phenobarbital the positive control. However, different test methods and comparisons should be sought to support this conclusion.

Keywords: Diazepam, fireplace test, phenobarbital, traction test

Introduction

Induction of anesthesia is commonly defined as the administration of drugs approximately 1-2 hours before anesthesia to assist the process of anesthesia. Pre-medications can be given before general or local anesthesia through intravenous (IV), intramuscular (IM), or subcutaneous (SC) route. Pre-medication drugs used for this purpose are classified into sedatives, such as diazepam and phenobarbital, and non-sedative drugs such as atropine.[1] Generally, phenobarbital and diazepam can be delivered by IM injection. Meanwhile, the most frequently used injections for solutions or suspensions were SC, IP, and IV injections. IP injection is a simple technique because the large surface area of the peritoneal cavity and many blood vessels allow for rapid absorption.[2]

Phenobarbital is a known derivative of the barbiturate group that is used for hypnotic therapy and epiplepsy treatments. This agent has a long-acting period, and a small dose of this agent is used as general anesthesia in rats.[3] Another drug, diazepam, also has the same use as phenobarbital, and can be used as an anesthetic induction and antiepileptic therapy agent. Diazepam is widely used for anxiety disorder, insomnia, alcohol withdrawal, and short-term relief of anxiety symptoms.[4] Thus, diazepam has largely replaced phenobarbital for anxiety and sleep disorders treatment due to fewer side effects. Nonetheless, diazepam and phenobarbital have the same general side effect, sedation.[4] Diazepam is also used frequently as a positive control in behavioral studies in mice. Meanwhile, phenobarbital is commonly used as a positive control in study anesthesia in rodents.[3,5,6] The phenobarbital side effect is hepatotoxicity, which may affect the use of this anesthetics in the anesthesia that assess the effects on the liver.[7,8] These may limit the use of phenobarbital as a positive control. Alternative positive controls need to be studied.

A previous study by Marina demonstrated that diazepam has more sedative effects than anxiolytic effects in mice.[9] This finding may be

used as basic information to study diazepam use in experimental animal research. Studies regarding the sedative effect of diazepam in experimental animals are still scarce despite the fact that diazepam is potentially used as an alternative positive control in studies on anesthesia and as a pre-medication anesthesia in animal studies. The administration route is a critical factor for the availability of the drugs in plasma and may affect the pharmacodynamics. In experimental animal studies, IP injection is frequently used because of the simplicity of the technique and the minimum stress it causes for the animals under study.[10]

Studies regarding diazepam's effectiveness in various injection routes are also limited. At the same time, this type of study can be informative to other researchers, especially those involved in animal experiments. Further studies are needed to assess the effectiveness of diazepam sedative effects in experimental animals, which mainly consist of mice and rats, using various routes of administration. Hence, this study aimed to determine the duration and effectiveness of diazepam sedative effect when it was administred through IP injection in mice.

Methods

This experimental study was performed on 3-6 month old male Swiss-Webster mice (*mus musculus*) weighed 20–30 grams. This study was condocuted in the Faculty of Medicine of Universitas Muhammadiyah Prof. DR. HAMKA, Indonesia. The mice are maintained under standard conditions: a temperature of 26 to 28 °C, a 12-hour light/dark cycle, and standard humidity. Animal acclimatization was done for ten days before the test and they had free access to food and water in their cages. These animal experiments were under the supervision of veterinarians to ensure their health during the acclimatization period until the tests were performed. These experiment animals were eligible to undergo several tests in this study. The procedures of study were approved by the Animal Ethics Committee, Universitas Muhammadiyah Prof. Dr. HAMKA (Protocol No.KEPKK/FK/024/01/2022).

This animal research applied the ethical principles of 3R (replacement, reduction, and refinement), which also referred to the 5F (five freedom) of free from hunger and thirst; free from discomfort; free from pain, injury and illness; free to express normal and natural behaviors; and free from fear and distress. The sample size was calculated using the Federer formula, resulting in 27 mice for three groups (n=9) selected by random sampling. The mice were then assigned to group I (normal saline as negative control), group II (phenobarbital as positive control), and group III (intervention with diazepam). All interventions was done using IP injections.

The dose of 0.325 mg phenobarbital for a mouse weighed 20 g is equivalent to 100 mg phenobarbital dose in human and adjustable based on the weight. Thus, a diazepam dose of 0.0325 mg for 20 g mice is equivalent with 10 mg of diazepam in humans, which was the dose used in this study. The animals performed several tests such as traction test, fireplace test, and examination of pupil diameter. The traction test was conducted first after the acclimatization, and all of the animals were ensured to be eligible for the study. The test was started when the mice was confirmed to be under the sedation effect by touching them and observing their reponse. The sedation effect was confirmed when there was less or no response. The onset of the drugs may vary between mice.

The traction test aimed to observe muscle relaxation activities, which was performed by placing the mouse in an anterior body position, facing a horizontally stretched wire, and had its tail pulled up. The test animals that failed to re-establish at least one of its posterior limbs to reach the wire were considered under a sedative effect. The duration needed by each mouse to make re-establishment were measured using a stopwatch and recorded.

The fireplace tests were conducted three days after the traction tests were finished. The animal tests were considered under a sedation effect when they showed no response or less response. The fireplace test was used to observe decreased activities and sensitivity towards the environment. The test was done by placing the mouse individually in a cylindrical tube to assess the time needed by the mouse to get out of the cylinder. The duration of the mouse's attempt to escape the cylindrical tube was recorded by a stopwatch. The pupillary reflex was used to determine the sedation effect by observing the pupil diameter. The normal pupil diameter in mice is 2 mm. This test was conducted during the fireplace test.

The statistical product and service solution (SPSS) program was used to analyze the results. The Kruskal–Wallis test was used to measure the significance of differences among groups. A value of $p<0.05$ was considered to reflect a statistically significant difference.

Table 1 General Characteristics of Mice

Parameter	Group		
	Negative Control (NS)	Positive Control (Phenobarbital)	Diazepam
n	9	9	9
Body Weight (g)	32.9	33.2	32.8
Pupil Diameter (mm)	2	2	2
Onset:			
Traction Test (min)	0	15	5
Fireplace Test (min)	0	10	3

Table 2 Parameter Test Results of Sedation Effect

Parameter Test	Group		
	Negative Control (n=9)	Positive Control (n=9)	Diazepam (n=9)
Traction Test	2 seconds ± 0.5	5 seconds ± 1.5	7 seconds ± 1.8
Fireplace Test	2 seconds ± 0.5	80 seconds ± 72.8	500 seconds ± 141.4
Pupil Diameter	2 mm	1 mm	1 mm

Results

Test animals were weighed during the study period as presented in Table 1, showing no variation among the groups with no significant increase in body weight observed during the study. The pupil diameter before tests were homogenous among groups with a normal-sized diameter pupil for mice (2 mm). There was no statistically significant differences in the body weight between different groups. The onset of the drugs (phenobarbital and diazepam) in traction and fireplace tests were not far different, albeit may vary.

The animal tests performed in this study comprised of several different interventions based on the groupings. Parameters of these tests, i.e., traction test, fireplace test, and pupil diameter assessments, were measured and data on the results were collected, as presented in Table 3. The tests were conducted to determine the effect of sedation in test animals by referring to the test results.

The negative control group took an average of two seconds in the traction and fireplace tests while the positive control group took longer, by an average of five seconds and 80 seconds, respectively. The diazepam group presented a more prolonged sedation effect than the positive control by an average of seven seconds in the traction test and 500 seconds in the fireplace test (Table 2). The assessment of the pupil diameter in the negative and positive controls and diazepam group demonstrated an average of 2 mm, 1mm, and 1 mm, respectively (Table 2).

The statistical analysis showed abnormally distributed data ($p<0.05$) based on the result of the Shapiro-Wilk test. Table 3 shows the significant difference between the positive

Table 3 Statistical Analysis Results in Several Parameter Tests

Group	Traction Test	Fireplace Test	Pupillary Diameter
	p	p	p
NS with Diazepam	0.000	0.000	0.000
Phenobarbital with Diazepam	0.015	0.001	1.000

control and the diazepam group ($p<0.05$) in the traction test, which was also true for the fireplace test ($p<0.05$). Pupillary diameter was not statistically different between animals in the positive control and diazepam groups ($p>0.05$). Meanwhile, the negative control and diazepam groups showed a statistical difference in pupillary diameter (Table 3).

Discussion

A significance difference in the test parameters between animals in the positive control and diazepam groups indicates a decrease in the test animal acitivities. This may be due to the Central Nervous System (CNS) suppression.[11] Most sedative drugs has a pharmacokinetics nature of fat soluble, well absorbed, and is distributed to the brain. High lipid solubility drugs can quickly enter the CNS. Sedative drugs are metabolized by liver enzymes and excreted by the kidney. However, different metabolic rates is observed for each drug class. Diazepam, a benzodiazepine group drug with an anxiolytic action, hypnotic, muscle relaxation, and anti-convulsion, is used in anesthesia and for insomnia conditions.

Diazepam's potential to elevate GABA is achieved by opening the chloride channels leading to the hyperpolarization of membrane, inducing CNS depression, and sedative activity. The traction and fireplace tests are commonly used as the assessment parameters for the sedative effect in mice.[12,13] The traction test showed the length of time needed by the mice to turn around and fall. The sedative effect becomes significant if the mice take longer time to turn around. Meanwhile, the fireplace test shows the length of time needed by the mice to jump out of the tube. The sedative effect gets stronger if the mice take longer time to jump out of the tube. The pupillary diameter changes may show decreased spontaneous activities as the consequences of a sedative effect.[14]The onset of drugs varied among groups, and could be affected by the route of administration. The intraperitoneal injection was selected to avoid potential degradation or modification of the drugs[2] and because intravenous (IV) injection is hard to use in rodents. The IP injection is minimally used in clinics, but preferred in animal studies. A study by Durk and colleagues shows that IP administration resulted in lower Tmax and higher Cmax than the subcutaneous (SC) injection.[15]

The traction and fireplace test results in the Diazepam group presented longer time than the positive control (phenobarbital), which may be because the benzodiazepine group (diazepam) becomes an active metabolite with a long half-life, resulting in a more prolonged sedative effect, while barbiturates, particularly phenobarbital, are partly excreted in the urine, with some extensively metabolized.[16]

A study by Sadanandan demonstrates that diazepam produced a longer duration of sleep than Ganaxolone.[17] Diazepam is frequently used as a comparator drug in studies related to sedative effect and anesthesia (intravenously). When used in anesthesia, diazepam is mainly combined with other agents.[16] In experimental animal models, benzodiazepines and older sedative-hypnotic drugs can exert an anti-anxiety effect. However, not all sedative drugs have this effect.[16] Furthermore, the sedative-hypnotic drug group are dose-dependent and induce sleep in a high dose. The specific drug and administration frequency could affect the sleep stages in the sedative-hypnotics effect.[16] Phenobarbital has a long duration of action and long half-life (80–120 hours), although the traction and fireplace test results showed a shorter duration of sedative effects in phenobarbital when compared to diazepam. Phenobarbital has low lipid solubility, protein binding to albumin at approximately 55%, and a long onset delay[18]while diazepam has a 20-80 hours half-life, highly lipid soluble and highly protein bound, and easily crosses the blood-brain barrier. Diazepam is considered a better choice due to its rapid onset and long half-life compared to phenobarbital or other drugs in the benzodiazepine group.[19]

In conclusion, diazepam has a better sedative effect than phenobarbital as it produces more prolonged sedative effect. Thus, diazepam can be used as an alternative agent for anesthesia in animal studies or as a positive control in an anesthesia study. Nevertheless, futher studies are need as no blood sample was collected to measure drug plasma concentration and organ assessment was not done to assess molecular parameters of sedative effect. These could be used as complementary data to resulting comprehensive results. This study followed the ethical principle of animal welfare, with the sample size calculated based on the Federer formula, and by using established methods. The health of the study animals was also ensured by having expert supervision. Further studies should be performed with different test methods, parameters, and comparisons.

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Original Article

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Association between Maternal Hemoglobin Level and Incomplete Abortion in A West Java Tertiary Hospital, Indonesia

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Abstract

Objective: To evaluate the association between maternal hemoglobin concentrations and incomplete abortion.

Methods: An analytic, cross-sectional study with consecutive sampling method was conducted using medical records of 45 pregnant women aged 18–35 years old visiting the Obstetrics and Gynecology Department of Dr. Hasan Sadikin General Hospital, Bandung, Indonesia from January 1, 2017 to December 31, 2019. Participants were grouped into incomplete abortion and non-abortion groups.

Results: Maternal characteristics in the incomplete abortion group showed that the majority of pregnant women in this group were 25.58 years of age, non-anemic (n=37, 82.22%), had no previous spontaneous abortion (n=40, 88.89%), and were nulliparous (n=25, 55.55%) with a mean interpregnancy interval of 4.03 years. The characteristics in both incomplete abortion group and non-abortion group were homogenous in the level of anemia (p-value=0.380), previous spontaneous abortion (p-value=1.00), and interpregnancy intervals (p-value=0.667). The mean hemoglobin concentration for the incomplete abortion group was 11.81 gr/dL (95% CI, 11.30 to 12.26). Heterogenous data was found in age (p-value=<0.001) and parity (p-value=0.002). Parity was a strong confounder, causing the hemoglobin concentration insignificantly associated to incomplete abortion (p-value=0.884).

Conclusion: No statistically significant association is found between hemoglobin concentration and incomplete abortion. Most women with incomplete abortion are around 25 years old, nulliparous, non-anemic with a mean hemoglobin concentration of 11.81 gr/dL with no history of previous abortion, and a rather secure interpregnancy intervals.

Keywords: Anemia, hemoglobin, incomplete abortion, interpregnancy intervals, parity

Introduction

Incomplete abortion is partial expulsion of conception or placenta weighing 500 grams or less with some parts left inside the uterus at gestational age before 20 weeks.[1] The incidence of spontaneous abortion is estimated at 23 million each year worldwide.[2] Causes of spontaneous abortion is multifactorial, primarily due to chromosomal anomalies.[3] In Indonesia, according to a meta-analytic study, the most prominent factors are maternal age and parity.[4] Other factors are education, early menarche, interpregnancy intervals, anemia, previous abortion, infections obesity, and hypertension.[3,4]

Anemia in pregnancy is a condition in which pregnant women have a hemoglobin (Hb) concentration below 11 gr/dL.[5] This condition can be due to hemodilution, excretion of iron through sweat, inadequate intake, heavy menstruation, malaria or parasitic infection, and anemia before gestation.[3] The World Health Organization (WHO) stated that the global prevalence of anemia among pregnant women aged 15 to 49 years is 36.5% in 2019.[6] In Indonesia, the national prevalence for this condition is 48.9% or 5 out of 10 pregnant women suffer anemia in 2018.[7] A study by Judistiani *et al*.[8] in West Java, Indonesia, in 2020 followed first trimester pregnant women. Their findings demonstrated an increased in anemia in the first trimester from 7.5% to 8.48%. This is concerning as anemia is linked to premature birth, low birth weight, preeclampsia, infections, and cardiac failure, which ultimately increases the mortality and morbidity rates of mothers and their infants.[3,9]

Some theories have presented a correlation between anemia and decreased oxygen supply or iron for the development of fetus, increased oxidative stress production and infection risk, and dysfunctional iron-dependent thyroid peroxidase.[10,11] A large-scale study involving almost 4 million women in China shows a significant association between severe anemia (<7 gr/dL) and an increased risk of abortion, while mild anemia (<11 gr/dL) is protective towards abortion.[10] Despite the facts that various studies had been performed regarding anemia and spontaneous abortion, they are rarely specifically connected with incomplete abortion or using categorized hemoglobin concentration. This study aimed to evaluate the association between maternal hemoglobin concentration and incomplete abortion.

Methods

An analytic observational study with cross-sectional design was performed on secondary data from medical records of pregnant women diagnosed with incomplete abortion at a gestational age of <20 weeks treated at the Department of Obstetrics and Gynecology, Dr. Hasan Sadikin General Hospital Bandung, West Java, Indonesia, from January 1, 2017 to December 31, 2019. The ethical clearance for this study was obtained from the Research Ethic Committee of Universitas Padjadjaran Bandung (no. 1155/UN6.KEP/EC/2022) and Dr. Hasan Sadikin General Hospital Bandung (no. LB.02.02/X.2.2.1/292/2023).

The inclusion criteria used were pregnant women in the age group of 18–35 years old; diagnosed for incomplete abortion as confirmed by ultrasound results, and had hemoglobin concentration recorded when patient first came to the emergency room of the hospital. Participants were excluded if data were incomplete data, such as patients' age, gestational age, and recorded hemoglobin concentration; had a history of diabetes mellitus, thyroid dysfunction, and hypertension; had multiple gestation; and had a history of lupus, antiphospholipid syndrome, and thalassemia. Data that were required in this study were age, interpregnancy intervals, and hemoglobin concentration as numerical variables; abortion incomplete, concluded level of maternal anemia, history of previous abortion, and parity as categorical variables. Consecutive sampling technique was used with a minimum sample of 35 for each incomplete abortion and non-abortion groups, based on a calculation using the unpaired numeric formula based on the hemoglobin concentration in a study by Guo *et al*.[12]

Diagnosis of incomplete abortion were confirmed by ultrasound. Data on hemoglobin concentration on admission were obtained from medical records for incomplete abortion cases. The Hospital Information System was used to obtain data for non-abortion cases from women with spontaneous delivery in the same time period. The data presented in mean, median, standard deviation, minimum, maximum values, and further categorized using the classification of anemia according to the WHO into non-anemia (Hb ≥11 gr/dL), mild anemia (Hb 10–10.9 gr/dL), or moderate anemia (Hb 7–9.9 gr/dL).[5] Cases were also categorized by parity based on past delivery at gestational age ≥24 weeks. The categories used were nulliparity (0 child), primiparity (1 child), and multiparity (≥2 children). Qualified data were processed using Microsoft Excel and IBM® SPSS® 26.

Univariate analysis was performed to analyze the subjects' characteristics. Data on hemoglobin concentration were tested for its normality using the *Kolmogorov-Smirnov* test. Then, a bivariate analysis with a confidence interval (CI) of 95% was used to test the hypothesis on the association of maternal hemoglobin concentration and incomplete abortion. The *Chi Square* test was used for anemia level and parity, whereas the *Fisher-exact* test was used to analyze the number of previous spontaneous abortions. Results of these analysis were considered as statistically significant and interpreted as having a cause-

effect association if the p-value ≤0.05.

Results

To obtain participants, consecutive sampling was applied on secondary data registry of patients treated from Janury 1, 2017 to December 31, 2019 and 45 samples each were selected for the incomplete abortion and non-abortion groups.

Maternal characteristics (Table 1) showed a mean age of 25.58 years old for the incomplete abortion group. A high number of cases of non-anemia was found in both incomplete abortion and non-abortion groups. Mild and moderate anemia cases was higher among non-abortion group, albeit not significantly different. Most subjects in both groups had no previous history of spontaneous abortion. The mean interpregnancy intervals in the incomplete abortion group and non-abortion groups were 4 years and 3.45 years, respectively. Both groups had a high number of nulliparous women, which was significantly higher in the non-abortion group. Both groups were found to have similar comparable baseline characteristics in terms of anemia level (p-value=0.380>0.05), number of previous spontaneous abortion (p-value=1.00>0.05), and interpregnancy intervals with a p-value of 0.667 (>0.05). However, for the parity (p-value 0.002<0.05) and age (p-value <0.001<0.05), the two groups were not similar. The *Post-hoc* test was then performed to each parity category, resulting in 1.00>0.05, 0.833>0.05, and 1.00>0.05 for nulliparity, primiparity, and multiparity, respectively. This showed that heterogeneity of parity in the compared groups was significantly associated with the association of hemoglobin concentration and incomplete abortion. This means that the number of parity could confound and cause no significant association between hemoglobin concentration and incomplete abortion.

In the incomplete abortion group, the mean and median hemoglobin concentrations were 11.81 gr/dL and 11.80 gr/dL, respectively, while the same values for non-abortion group were 11.90 gr/dL and 12.20 gr/dL. To define the association between the hemoglobin concentration level and incomplete abortion, an analysis was conducted after the normality test using the non-parametric independent-sample analysis of *Mann-Whitney U* test due to the fact that the data of the abortion group and non-abortion groups were not

Table 1 Study Population Characteristics

Characteristics	Incomplete Abortion (n=45)	Non-Abortion (n= 45)	p-value
Age (years)			
Mean (SD)	25.58 (4.59)	29.16 (4.79)	<0.001
Anemia Level, n (%)			
Non-Anemia	37 (82.22%)	32 (71.11%)	
Mild Anemia	2 (4.45%)	5 (11.11%)	**0.380**
Moderate Anemia	6 (13.33%)	8 (17.78%)	
Number of Previous Spontaneous Abortion, n (%)			
0	40 (88.89%)	40 (88.89%)	**1.00**
1	5 (11.11%)	5 (11.11%)	
Interpregnancy Intervals (years)			
Mean (SD)	4.03 (2.86)	3.45 (3.08)	0.667
Parity, n (%)			
Nulliparity	25 (55.55%)	40 (88.89%)	
Primiparity	12 (26.67%)	1 (2.22%)	0.002
Multiparity	8 (17.78%)	4 (8.89%)	

Table 2 Association between Hemoglobin Concentration and Incomplete Abortion

Variable	Hemoglobin Concentration (gr/dL)		95% CI	p-value
	Mean (SD)	Median (range)		
Incomplete Abortion	11.81 (1.53)	11.80 (7.60-14.70)	11.30 – 12.26	0.884
Non-Abortion	11.90 (1.68)	12.20 (8.30-14.70)	11.31 – 12.31	

normally distributed. This analysis resulted in a p-value of 0.884 (>0.05), demonstrating no statistical significant association between the hemoglobin concentration and incomplete abortion (Table 2).

Discussion

It was revealed in this study that the maternal hemoglobin concentration in early pregnancy is not associated with incomplete abortion. Hemoglobin concentration in the incomplete abortion group presents a lower mean level of 11.81 gr/dL when compared to the non-abortion group (11.90 gr/dL). A similar difference in concentration is also reported by Guo *et al*.[12] among women treated at the Beijing Obstetrics and Gynecology Hospital, in which first trimester pregnant women have a higher mean hemoglobin concentration level of 13.22 gr/dL as opposed to women with spontaneous abortion (12.59 gr/dL). A study by Díaz-López *et al*.[11] in 2021 and Xu *et al*.[10] in 2020 do not correspond to the findings of the present study by showing that an increased risk of spontaneous abortion is found in women suffered from severe anemia (p-value=<0.001; OR, 1.52; 95% CI, 1.25 to 1.86), low concentration (Hb <11 gr/dL; *p*-value=0.002<0.05) and high concentration (Hb>14 gr/dL; p-value=0.012<0.05).

The identified association might be due to the fact that women were hemorrhaging for hours to days before hospital admittance, as well as the presence of strong confounding factors of parity and age, and/or other factors that were not established in current study. Moreover, blood test result data was based on the the data at patient admission. The very first antenatal care (ANC) hemoglobin data were not available as most patients had ANC outside the area of study and some may not even seek any care at all. Although current study showed that most pregnant women in first trimester have hemoglobin concentration above the lower cutoff of 11 gr/dL determined globally, this number is still lower compared to other pregnant women outside of Indonesia. This might be because of differences in the demography, climate, lifestyle, and health-seeking behaviors among different countries. A low hemoglobin level during adolescence (aged 10-18 years) is said to be carried over to adulthood and contribute to anemia in pregnancy; hence, it is important to prepare early and reach the adequate hemoglobin level in preconception stage.[13]

The mean age of women in the incomplete abortion group is 25 years, which is younger than those in the non-abortion group. A study in Norway has shown an absolute lowest risk of spontaneous abortion in 27 year-old pregnant women (range, 25–29 years old).[14] A case-control study by Yanti L.[15] has demonstrated that maternal age is significantly correlated positively with spontaneous abortion with a *p*-value of <0.01 (r=0.297), despite the fact that another study found no significant correlation (OR, 1.587; *p*-value=0.202<0.05).[16] Findings in the current study might show that women with more mature age have a more solid plan to conceive; therefore they are more open in finding supports or information, restricting their daily activities, and seeking more care or more likely to take supplements in order to achieve a successful pregnancy.

Most women in the incomplete abortion group fall into non-anemia category, which corresponds to the findings of a study done in Bahagia general hospital in Makassar City, Indonesia. However, this is different from a study done in Kediri district, Indonesia, in 2017, where the highest occurrence of abortion is seen in subjects with severe anemia (59.5%; p-value=0.000; r=0.504).[17,18] The non-anemic state presented by most subjects might suggest that they had received adequate care, such as taking Fe or multivitamins, consuming nutritious food, and applying healthy lifestyle. Still, hemoglobin concentration alone could not be accounted for adequate iron stores.

Several mechanisms caused by anemia and iron deficiency in pregnancy may also lead to spontaneous abortion. Hypoxic state due to decreased oxygen supply could stimulate fetal production of cortisol that will disturb the fetal development and estrogen-progesteron function, leading to myometrium contraction and cervix dilation.[11,19] Increased oxidative stress could disturb trophoblast invasion and the development of spiral arteries which were not yet embedded firmly.[18] Therefore, explained the early expulsion of conception.

Most subjects with incomplete abortion present no previous history of spontaneous abortion (88.89%). This finding is similar to the findings of a study performed in the same region of Bandung city that showed a higher number of subjects withouth previous abortion history in the incomplete abortion group with an insignificant correlation (56.98%; p-value=0.111).[20] Another study by Arnianti *et al*.[21] in 2021 had different results which abortion group with previous abortion had percentage of 56.5% and increased risk of 2.97 times (95% CI, 1.05 to 8.37) for subsequent abortion. A history of spontaneous abortion is not a predicting factor that the subsequent pregnancy would end in another abortion despite a 20% increase in the risk for abortion.[3] It is speculated that history of spontaneous abortion could cause trauma, both physically and psychologically. Curettage could scar the endometrium which will lead to a suboptimal condition for the fetus and placenta to grow. The preceding loss could lead to stress, substance abuse, and unhealthy lifestyle that could put current pregnancy at risk.

In terms of the interpregnancy intervals, the mean interval for subjects with incomplete abortion is 4 years, ranging from 4 months to 9 years. A case-control study by Purwaningrum *et al*.[22] stated that pregnant women with interpregnancy intervals of less than 6 months or more than 48 months have a four-fold increased risk (OR, 4.2; *p*-value=0.01<0.05) of spontaneous abortion than those with an interval between 6 to 48 months. Mremi *et al*.[23] in 2022 found that interpregnancy intervals is significantly correlated with post-partum anemia and is 10 times higher in women who have less than a 2-year interval between pregnancies. Pregnancy had been progressively using maternal iron stores for the development of the fetus in each trimester, hence it would be deficient by the end of the term. It was said that the body needed at least eight weeks to return red blood cells volume and hematocrit to normal, not to count other events that could make the wait time longer, such as anemia during pregnancy, blood loss during delivery, post-partum hemorrhage, and lochia that happened for weeks.[3] A big gap between pregnancies (>5 years) was also considered to be a risk due to an increased in maternal age and the need for the uterine wall to adjust as if it was the first pregnancy. Hence, preconception counseling and supplementations became important to anticipate such factors that could contribute to spontaneous abortion.

Incomplete abortion most often occurred in nulliparity or women who were pregnant for the first time (55.56%). These findings corresponded to a study done in Mojokerto in 2022 which stated that first pregnancy had risk for abortion (42.4%).[24] Different results were reported from a study in Al-Ihsan General Hospital Bandung in 2020 that multiparity had higher number (66.67%) among women with incomplete abortion and more number of parity was causing higher proportion of abortion (*p*-value=0.08<0.05).[20] This study, supported by another study in Nepal showed that parity was a significant predictor of hemoglobin concentration, as well as maternal age.[25] Increasing parity numbers could lead to decrease hemoglobin concentration and serum ferritin in the subsequent pregnancy due to inadequate replenishment from the preceding spent of iron stores, which was also influenced by interpregnancy intervals.[4]

Thus, this study discovered no significant association between inclomplete abortion and maternal hemoglobin concentration, and that parity could be a strong confounder. The prevalence of anemia in pregnant women treated in Dr. Hasan Sadikin General Hospital Bandung is low; hence may explain the non-association. This study also has several limitations. Most subjects are not anemic and study populations are quite different as data for incomplete abortion were taken from medical records and the non-abortion data were collected from SIRS. Hemoglobin concentration data from the very first antenatal care cannot be acquired as subjects received care outside the study location. Limited time and being single-centred have made the sample study small and might not accurately reflect the actual condition. Future studies are suggested to involve multicenters and a longer period of time in the hope that more precise results can be obtained. It is recommended to do analysis solely among pregnant women with anemia.

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