Online ISSN: 2249-4618
Print ISSN: 0975-5888

Global Journal of Medical Research: K

Interdisciplinary

Hypoxia Disorders

Prevalence of Bovine

Highlights

Polymorphism in Bronchogenic

Enzyme Nicotinamide Adenine

Discovering Thoughts, Inventing Future

VOLUME 14 ISSUE 3 VERSION 1.0

Global Journal of Medical Research: K
Interdisciplinary

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Interdisciplinary
Volume 14 Issue 3 (Ver. 1.0)

Open Association of Research Society

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

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USA Tax ID: 98-0673427

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Global Journal of Medical research: K
Interdisciplinary
Volume 14 Issue 3 Version 1.0 Year 2014
Type: Double Blind Peer Reviewed International Research Journal
Publisher: Global Journals Inc. (USA)
Online ISSN: 2249-4618 & Print ISSN: 0975-5888

Role of Detoxifing Enzyme Nicotinamide Adenine Dinucleotide (Phosphate) H: Quinone Oxidoreductase-1 C609T Gene Polymorphism in Bronchogenic Carcinoma

By Mohamed Moustafa Rezk, Ahmed Youssef Shaaban, Nermine Hossam Elden Zakaria, Reham Fadl Moftah & Shaima'a Mahmoud Okasha

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Abstract- Lung cancer is currently one of the most common cancers and a major cause of cancer-related death in the world. Eighty-five percent of lung cancers are non-small cell lung cancers (NSCLCs), and 15% are small cell lung cancers (SCLCs). The most important risk factor for lung cancer is tobacco smoking. Polycyclic aromatic hydrocarbons (PAHs) are abundant in tobacco smoke and constitute a major etiological factor in lung cancer. NAD (P) H: quinone oxidore - ductase (NQO1) is a cytosolic flavoprotein that catalyzes the two-electron reduction of quinoid compounds into less toxic hydroquinones. A single base substitution (C→T) polym - orphism at 609 in the NQO1 gene reduces quinone redu - ctase activity. Published data on the association between NQO1609 C>T polymorphism and lung cancer risk are conflicting. In this study, we investigated NQO1genotype in relation to lung cancer risk. The cases were patients attending Chest diseases unit in the Alexandria Main University Hospital with bronchogenic carcinoma in different stages. The control group consisted of age-matched male adults from the same socioeconomic class. DNA extraction from EDTA blood samples and genotyping was successfully carried out for 100 cases and 100 controls by PCR-RFLP and PCR-CTPP.

GJMR-K Classification: NLMC Code: QZ 365

Strictly as per the compliance and regulations of:

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Mohamed Moustafa Rezk [α], Ahmed Youssef Shaaban [σ], Nermine Hossam Elden Zakaria [ρ], Reham Fadl Moftah [ω] & Shaima'a Mahmoud Okasha [¥]

Abstract- Lung cancer is currently one of the most common cancers and a major cause of cancer-related death in the world. Eighty-five percent of lung cancers are non-small cell lung cancers (NSCLCs), and 15% are small cell lung cancers (SCLCs). The most important risk factor for lung cancer is tobacco smoking. Polycyclic aromatic hydrocarbons (PAHs) are abundant in tobacco smoke and constitute a major etiological factor in lung cancer. NAD (P) H: quinone oxidore - ductase (NQO1) is a cytosolic flavoprotein that catalyzes the two-electron reduction of quinoid compounds into less toxic hydroquinones. A single base substitution (C→T) polym - orphism at 609 in the NQO1 gene reduces quinone redu - ctase activity. Published data on the association between NQO1609 C>T polymorphism and lung cancer risk are conflicting. In this study, we investigated NQO1genotype in relation to lung cancer risk. The cases were patients attending Chest diseases unit in the Alexandria Main University Hospital with bronchogenic carcinoma in different stages. The control group consisted of age-matched male adults from the same socioeconomic class. DNA extraction from EDTA blood samples and genotyping was successfully carried out for 100 cases and 100 controls by PCR-RFLP and PCR-CTPP. Pat - ients carrying at least one variant allele for the NQO1 609 SNP (CT/TT genotype) were found to have almost a 2.2 -fold increased lung cancer risk than those with CC genotype, 4.3-fold increased risk of developing SCLC and 3.8- fold increased risk for lung cancer with other histological type. Furthermore, the heavy smokers (>21 p-y) patients with one or two copies of the T variant allele had 3.6-fold increased lung cancer risk compared to those with CC genotype, while, the risk for squamous cell carcinoma was 2.4-fold and 17.9-fold for SCLC. These results suggest that individuals with reduced enzyme activity, due to NQO1 609 C>T polymorphism, may therefore have an increased risk of lung cancer.

I. Introduction

Lung cancer is currently one of the most common cancers and a major cause of cancer-related death in the world. Among males, the highest lung can - cer incidence rates are in Central and Eastern Europe 53.5 (per 100,000) and 50.4 in Eastern Asia. Among females, the highest lung cancer incidence rates are 33.8 in Northern America and 23.7 Northern Europe. [(1)]

Incidence data (ASR) for the Arab countries; for males, lung cancer incidence estimated as; 31.1 (per 100,000) in Tunisia followed by Lebanon, Libya, Jordan and in Egypt 11.21 (per 100,000). For females, estim - ated as; 11.0 in Lebanon followed by Bahrain, Syrian Ar -ab Rebuplic, United Arab Emirates, and in Egypt it reaches 3.76 (per 100,000).[(1)]

Eighty-five percent of lung cancers are non-small cell lung cancers (NSCLCs), and 15% are small cell lung cancers (SCLCs).[(2)]

The most important risk factor for lung cancer is tobacco smoking. International variations in lung cancer rates and trends largely reflect differences in the stage and degree of the tobacco epidemic because smoking accounts for about 80% of global lung cancer deaths in men and 50% of the deaths in women.[(3)]

Many of the compounds in tobacco smoke are oxidized by phase I enzymes into reactive metabolites, which are detoxified by phase II enzymes. Polycyclic aromatic hydrocarbons (PAH) are abundant in tobacco smoke and constitute a major etiological factor in lung cancer.[(4)]

NQO1 [NAD (P): H-(quinone acceptor) oxidore ductase; EC 1.6.99.2] [(5)] enzyme is a homodimeric flavin adenine dinucleotide (FAD) containing cytosolic protein catalyzing the two-electron reduction of quinone subs - trates.[(6)] Quinine compounds are mainly derived from endogenous quinones, such as vitamin E quinine, and exogenous quinones, such as exhaust gas, tobacoo smoke.[(7)]

NQO1 prevents the generation of free radicals and reactive oxygen, thus protecting the cells from oxidative damage. This pathway is thought to be the major mechanism responsible for modifying the toxicity of quinones, including those arising from the formation of DNA adducts induced by benzo(a)pyrene 3,6-quin - one, one of the most potent polycyclic aromatic hydro - carbons present in tobacco smoke.[(8)]

The NQO1gene is located on chromosome 16q22.1, spanning 17.2 kb and consisting of 6 exons

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and 5 introns.[9] The polymorphic variant is a C→T transition at nucleotide position 609 (amino acid codon 187) that results in a proline-to-serine amino acid subs - titution in the protein. The reference number of this SNP in the database of the National Center for Biotechnology Information (NCBI) is rs1800566.[10]

Three genotypes of NQO1 are known to be associated with different enzymatic activities: C/C is the homozygous wild-type with normal activity, C/T is heter - ozygous with reduced activity, and T/T is the homoz - ygous variant with only 2–4% of the enzyme activity of the wild-type.[11]

Several studies have examined the relationship between the NQO1 genetic polymorphism and lung cancer risk, but the conclusions have been inconsistent.[9,12,13]

The study of the role of SNPs and in particular the NQO1 609C>T polymorphism as a risk factor for the development of bronchogenic carcinoma among Egy - ptian male patients is of utmost importance for the unmasking of the risk factors underlying bronchogenic carcinoma in Egypt.

II. Patients and Methods

The current study was conducted as a hospital-based case-control study of 200 subjects; 100 male patients presenting to the Chest diseases unit in the Alexandria Main University Hospital with bronchogenic carcinoma in different stages of the disease between 2011 and 2013. Lung cancer cases were newly diagnosed cases aged above 40 years old with absence of previous precancerous conditions and any known primary cancerous lesion elsewhere. Diagnosis of primary lung cancer was confirmed through a review of each patient's pathology report by the Alexandria University Hospital pathology department. One hundred age-matched male adults from the same socioe - conomic class were recruited. All patients and controls provided informed consent.

a) DNA extraction and genotyping analysis

DNA was extracted from EDTA whole blood using DNA purification kit (QIAamp DNA blood Mini Kit, Qiagen, Hilden, Germany). The extraction was done according to the manufacturer's instructions using the Spin Protocol. The quantity and purity of DNA for each sample was assayed using Nanodrop 2000 spectrop - hotometer (Thermo scientific, USA). The final conce - ntration of target DNA was adjusted to 50-100 ng in the following amplification reaction to exclude variability in DNA concentration.

DNA amplification was conducted by polym - erase chain reaction with confronting two-pair primers (PCRCTPP) [14] , using four primers; F1: 5'-CCT TAT CAGAGT GTC TTA CTG AGA-3' (54.4°C) and R1: 5'-CAA TGCTAT ATG TCA GTT GAG G-3' (54.7°C), for C allele amplifying a 165-bp band, as well as F2: 5'-GTG GCT TCC AAG TCTTAG AAT-3' (54.9°C) and R2: 5'-TTT CTA GCT TTG ATC TGGTTG-5' (54.5°C) for T allele amplifying a 283-bp band. A common 406-bp band was designed to be amplified between primers F1 and R2.

The results of PCR-CTPP genotyping were confirmed by PCR-RFLP with HinF1 enzyme, which produces 188-bp and 85-bp bands for C allele and 151-bp and 85-bp bands for T allele using primers F: 5'-AGT GGCATT CTG CAT TTC TGT G-3', and R: 5'-GAT GGA CTT GCCCAA GTG ATG-3'. (14) Two hundred samples were successfully genotyped;

100 lung cancer patients (62 were CC, 31 were CT, and 7 were TT) and 100 healthy individuals as a control group (73 were CC, 26 were CT, and 1 was TT), The results of PCR-CTPP were consistent with those of the gold standard method PCR-RFLP.

b) Statistical analysis

All statistical analyses were performed using the SPSS20.0 program. An effect was considered statis - tically significant at $P<0.05$. X2 test was cond -ucted for examining the Hardy-Weinberg equilibrium and indepe - ndence of genotype frequency between cases and controls. Logistic regression analysis was then perfo - rmed to compute odds ratios and 95% confidence inte - rvals, after adjustment for age, family history and smoking index pack-years (number of years smo - ked×number of packs smoked per day).

III. Results

The study population was Egyptians, they consisted of 100 male lung cancer patients and 100 male of age- matched healthy controls. The mean age was 54.2 ± 8.2 years for cases and 52.7 ± 8.4 years for controls. There was a statistically significant difference between the cases and controls concerning the smo - king status and smoking index ($p<0.001$).Cases were more likely than controls to be current smokers. The mean Pack-Years were 43.9 ± 20.8 among cases and 19.5 ± 14.3 among controls. Twenty three (23%) of the patients had positive family history of cancer compared to 4% of the control group with a statistically significant difference ($p<0.001$).

In the group of lung cancer patients, the most prevalent histological subtype of lung cancer was Aden - ocarcinoma with a frequency of 37%, followed by 31% squamous cell carcinoma, 19% SCLC (small cell lung cancer) and 13% with other histological subtypes (7 cases were large cell anaplastic carcinoma, 4 cases were undifferentiated NSCLC and 2 cases were carci - noid tumor). As regards the tumour staging, 5 out of 100 patients were stage I, 27 patients were stage II, 32 patients were stage III and 36 patients were stage IV.

All included subjects were analyzed for the NQO1 609C>T (rs1800566) SNP genotype. The distrib - utions of the NQO1 genotypes among lung cancer patients and controls were in agreement with Hardy-

Weinberg equilibrium (p= 0.267, 0.425, respectively). The frequency of NQO1 CC, CT, TT genotypes was 62, 31 and 7%, respectively in patients and 73, 26 and 1% in controls. The relative frequencies of the wild (C) allele and the variant (T) allele of NQO1 609C>T (rs1800566) SNP were 77.5% and 22.5%, among the patients respe - ctively, and were 86% and 14% among the controls, respectively. There was a significant difference among the two groups as regards their allele frequencies (p =0.028).

Table 1 : Comparison between patients and controls according to genotypes and allele frequencies

	patients (n=100)		Controls (n=100)		χ^2	p
	No.	%	No.	%		
Genotype						
CC	62	62.0	73	73.0		
CT	31	31.0	26	26.0	5.835*	$^{MC}p = 0.047$*
TT	7	7.0	1	1.0		
Allele						
C	155	77.5	172	86.0	4.843*	0.028*
T	45	22.5	28	14.0		

*χ^2: value for Chi square *: Statistically significant at $p \leq 0.05$*

Patients with one or two copies of the T variant allele had 2.2 -fold increased lung cancer risk than those with CC genotype (adjusted OR=2.2; 95%CI: 0.63-7.9). To assess whether the NQO1 variant T allele may impart different risks for the various lung cancer cell types, we computed the adjusted ORs for lung cancer for each histological type. Patients with one or two copies of the T variant allele had 4.3- fold increased SCLC risk than those with CC genotype (adjusted OR=4.3; 95%CI: 0.57-33.4), while, they had 3.8- fold increased risk for lung cancer with other histological type (adjusted OR=3.8; 95%CI:0.49-30.7), 2.1-fold increased adeno - carcinoma risk (adjusted OR=2.1; 95%CI: 0.43-10.3), and 1.2-fold increased squamous cell carcinoma risk more than those with CC genotype (adjusted OR=1.2; 95%CI: 0.21-7.0).

Table 2 : Stratified analysis for NQO1 609C>T (rs1800566) SNP by histological type of the tumor among cases and controls

	Genotype						OR (95% CI)[a]	
	CC		CT		TT		Crude	Adjusted#
	No	%	No	%	No	%		
Controls	73	54.1%	26	45.6%	1	12.5%	1	1
All cases	62	45.9%	31	54.4%	7	87.5%	1.7 (0.91-3.0)	2.2 (0.63-7.9)
SCC	19	20.7%	8	23.5%	4	80.0%	1.7 (0.73-3.9)	1.2 (0.21-7.0)
SCLC	9	11.0%	10	27.8%	0	0.0%	3.0 (1.1-8.2)*	4.3 (0.57-33.4)
Adeno-carcinoma	25	25.5%	10	27.8%	2	66.7%	1.3 (0.57-2.9)	2.1 (0.43-10.3)
Others	9	11.0%	3	10.3%	1	50.0%	1.2 (0.34-4.2)	3.8 (0.49-30.7)

[a]Odds ratios are for CT and TT versus CC.
 # OR adjusted for age, family history, smoking index OR significant at 0.05*

The study subjects were stratified by age and smoking status. As regards study subject's age, elevated lung cancer risk associated with TT genotype was evident in younger individuals (age <60) (adjusted OR=13.6; 95%CI: 0.55-58.7). On the contrary, elevated lung cancer risk associated with CT genotype individ - ually and with one or two copies of the T variant allele (CT and TT combined) were evident in older individuals (age >60) ((adjusted OR=9.1; 95%CI: 0.90-91.7), (adjusted OR=10.6; 95%CI: 1.1-58.7), respectively). The only significant OR was for the combined CT and TT genotype group above 60 years.

When adjustment was made for smoking index pack-years (light smokers and heavy smokers), among light smokers (<21 p-y), the patients with one or two copies of the T variant allele had 1.9-fold increased lung

cancer risk compared to those with CC genotype (adjusted OR=1.9; 95%CI: 0.29-13.2), while, the heavy smoker (>21 p-y) patients with one or two copies of the T variant allele had 3.6-fold increased lung cancer risk compared to those with CC genotype (adjusted OR=3.6; 95%CI: 0.41-30.7). The patients with one or two copies of the T variant allele had 3.6-fold increased lung cancer risk compared to those with CC genotype (adjusted OR=3.6; 95%CI: 0.41-30.7), while, the risk for squamous cell carcinoma was 2.4-fold (adjusted OR=2.4; 95%CI: 0.21-27.9), 17.9-fold for SCLC (adju - sted OR=17.9; 95%CI: 1.5-40.6), 2.4-fold for adenoca - rcinoma (adjusted OR=2.4; 95%CI: 0.25-23.8), and 2.5-fold for lung cancer other histological type (adjusted OR=2.5; 95%CI: 0.18-34.4). The odds ratio of the combined SCLC heavy smokers group was statistically significant.

Table 3 : Comparison between NQO1 609C>T genotypes as regards smoking among patients

	Genotype						Test of sig.	p
	CC (n = 62)		CT (n = 31)		TT (n = 7)			
	No	%	No	%	No	%		
Smoking								
Non smoker	0	0.0	2	6.5	2	28.6		
Smoker	62	100.0	27	87.1	5	71.4	18.737*	^{MC}p = 0.001*
EX smoker	0	0.0	2	6.5	0	0.0		
p_1			0.012*		^{FE}p = 0.009*			
p_2			^{MC}p = 0.173					
Smoking intensity								
Min. – Max.	0.50 - 4.0		0.50 - 3.0		1.50 - 2.50		^{KW}	
Mean ± SD.	1.58 ± 0.63		1.39 ± 0.51		1.90 ± 0.42		5.436	0.066
Median	1.50		1.50		2.0			
$^{MW}p_1$			0.187		0.091			
$^{MW}p_2$			0.025*					
Duration of smoking (years)								
Min. – Max.	15.0 - 50.0		5.0 - 55.0		20.0 - 40.0			
Mean ± SD.	28.60 ± 9.99		27.93 ± 13.0		31.0 ± 7.42		^{KW} 0.367	0.832
Median	30.0		30.0		30.0			
$^{MW}p_1$			0.935		0.499			
$^{MW}p_2$			0.711					
Smoking Index (p/year)								
Min. – Max.	7.0 - 100.0		2.50 - 110.0		30.0 - 100.0			
Mean ± SD.	43.37 ± 17.28		41.91 ± 26.20		60.50 ± 25.27		^{KW} 2.873	0.238
Median	42.50		40.0		60.0			
$^{MW}p_1$			0.680		0.105			
$^{MW}p_2$			0.117					

p: p value for comparing between different genotype
p_1 : p value for comparing between CC with CT and TT
p_2 : p value for comparing between CT and TT
χ^2: value of Chi square
MC: Monte Carlo test *FE: Fisher Exact test*
$^{KW}\square^{\square}$: Kruskal Wallis test *MW: Mann Whitney test*
**: Statistically significant at $p \leq 0.05$*

Table 4 : Stratified analysis for NQO1 609C>T (rs1800566) SNP by smoking index among cases and controls

	Smoking index <21				OR (95% CI)		Smoking index >21				OR (95% CI)	
	CC		CT+TT		Crude	Adjusted #	CC		CT+TT		Crude	Adjusted #
	No	%	No	%			No	%	No	%		
Controls	13	76.5%	5	45.5%	1	1	8	12.1%	1	3.7%	1	1
All cases	4	23.5%	6	54.5%	3.9 (0.76-19.9)	1.9 (0.29-13.2)	58	87.9%	26	96.3%	3.6 (0.43-30.1)	3.6 (0.41-30.7)
SCC	3	18.8%	2	28.6%	1.7 (0.22-13.7)	1.2 (0.05-18.6)	16	66.7%	6	85.7%	3.0 (0.31-29.4)	2.4 (0.21-27.9)
SCLC	1	7.1%	0	0.0%	2.4 (0.25-14.7)	2.1 (0.21-25.9)	8	50.0%	9	90.0%	9.0 (1.0-88.5)*	17.9 (1.5-40.6)*
Adenocarcinoma	0	0.0%	2	28.6%	3.7 (0.16-10.6)	1.3 (0.07-16.8)	25	75.8%	9	90.0%	2.9 (0.32-26.4)	2.4 (0.25-23.8)
Others	0	0.0%	2	28.6%	3.7 (0.16-10.6)	1.4 (0.05-17.5)	9	52.9%	2	66.7%	1.8 (0.13-23.5)	2.5 (0.18-34.4)

 # OR adjusted for age and family history
** OR significant at 0.05*

Assessing the efficiency of CTPP-PCR considering allelic discrimination through genotyping analysis of NQO1 609C>T (rs1800566) SNP, comparing CTPP-PCR results with those of RFLP-PCR as a gold-standard method. The efficiency of CTPP-PCR was assessed in 200 samples; 100 lung cancer patients and 100 healthy individuals as a control group. There was no discrepancy between the CTPP-PCR and RFLP-PCR results in all our study subject samples. The previous performance reflects a sensitivity of 100% and specificity of 100%. The positive predictive value (PPV) and the negative predictive value (NPV) were 100%.

IV. Discussion

Cancer lung is one of the leading causes of cancer related mortalities worldwide. The rapidly evolving field of cancer genetics has opened up new possibilities for the discovery of susceptibility genes for numerous cancers including lung cancer.

Lung cancer has been the most common cancer worldwide since 1985, both in terms of incidence and mortality. The 5-year survival rate in the United States for lung cancer is 15.6 %. [15]

Cigarette smoking is the main risk factor for lung cancer, accounting for about 90% of the cases in men and 70% of the cases in women. [16] The pathogenesis of lung cancer had a genetic component, whether it relates to host susceptibility to lung cancer, with or without exposure to cigarette smoke to the development of certain types of lung cancer. [15]

NAD (P) H: quinone oxidoreductase 1 (NQO1) is a two-electron reductase, which reduces reactive quinones to less reactive and less toxic hydroquinones, resulting in protection of the cells. [13] The quinones are mainly derived from endogenous quinones, such as vitamin E quinine and exogenous quinones, such as tobacoo smoke. [17] The T/T (Ser/Ser) variable allele of NQO1 lacks enzymatic activity and fails to detoxify quinone metabolites into the reduced form. [13] It was thus hypothesized that individuals lacking NQO1activity would be at high risk of malignancies (lung cancer) because of the exposure to procarcinogens, that are included in cigarette smoke, which are oxidized to quinine metabolites. [6]

In this study, we analyzed the relationship between NQO1genetic polymorphisms and lung cancer, comparing lung cancer patients and healthy individuals in Egypt. Our results showed, the frequencies of the wild (C) allele and the variant (T) allele of NQO1 609C>T (rs1800566) SNP for the patients were 77.5% and 22.5%, respectively, and for the controls were 86% and 14%, respectively. The allele frequencies in both cases and controls were in Hardy–Weinberg equilibrium. The frequency of the variant allele in Egyptians was similar to the frequencies reported in another study on Arab population. [18]The NQO1 609C>T polymorphism exhibits ethnic variation (4–22%) with the highest prevalence of the T allele occurring in Asian populations and the lowest in Caucasians (4%), while, in Arabs TT genotype frequency was 6.4% (Middle Eastern Arab origin (95% Saudi Arabians and 5% from other Arab countries such as Jordan, Syria, Lebanon, Yemen) and data in this study are consistent with this frequency.[18, 19]

We found that, Egyptian Patients with one or two copies of the T variant allele had 2.2 -fold increased lung cancer risk than those with CC genotype. However, the patient with one or two copies of the T variant allele had a 4.3-fold increased risk for developing small cell lung cancer, 3.8-fold increased risk to lung cancer other than squamous cell carcinoma or adinocarcinoma and 2.1-fold increased risk of adenocarcinoma more than those with CC genotype.

Table 5 : Log-rank test comparing survival distributions among the three groups of the patients as regards NQO1 609C>T genotype

Overall Comparisons			
	Chi-Square	df	Sig.
Log Rank (Mantel-Cox)	5.010	2	.057
Test of equality of survival distributions for the different levels of Genotype.			

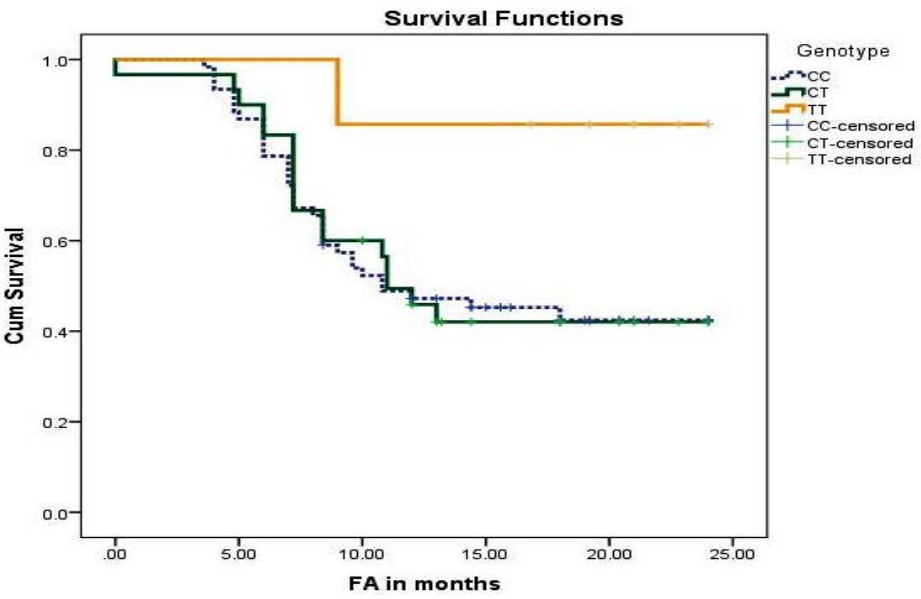

Figure I : Kaplan-Meier curve comparing estimated survival among the study lung cancer patients with CC, CT and TT genotype

Our results are supported by Lewis et al., [13] who reported the increased risk associated with genotypes containing at least one variant T allele seems to be restricted to SCLC only among Caucasians. In studies performed in Asians, the NQO1 CC genotype was found to be associated with lung cancer, particularly adenocarcinoma, [20] whereas the variant NQO1 variant T allele has been suggested to be a risk factor for lung cancer in Caucasians.[9,13,21] The only previous study on Caucasians with sufficient number of cases that performed an analysis of histologic subtypes was in agreement with our study that the variant NQO1 genotypes were overrepresented in squamous cell carcinoma. [21]

On the other side, in Asian population, the wild-type C allele was found with higher incidence among subjects with adenocarcinoma. [12, 20] Furthermore, the variant allele was found protective against adenoc - arcinoma in this population, while no such effect was observed in case of SCLC. [22]

To explore the connection between NQO1 609C>T (rs1800566) polymorphism and lung cancer risks, we stratified the data by age, smoking and family history among our study subjects. We observed a statistically significant 10.6-fold increased risk among older individuals (age >60) with even one variant T allele compared to 1.2-fold among younger individuals (age<60). However, the association between NQO1poly -morphism and lung cancer risk might differ depending on subject's age. Studies demonstrating the pro-carcinogenic effect of NQO1variant T allele in young Caucasians but rather protective effect in older ones can be found (age <50 years: OR = 1.28; age ≥50 years: OR = 0.46). [23]

In our study, the patients who were heavy smokers (>21 pack-years) and with one or two copies of the T variant allele had 17.9- fold increased risk for SCLC lung cancer than light smoker patients with 2.1-fold increased risk. However, the overall lung cancer risk among heavy smokers (>21 pack-years) 3.6-fold incre - ased risk compared to 1.9-fold among light smokers (<21 pack-years).

Xu et al. [21] found that both the C/T and T/T genotype produced a higher risk of lung cancer comp - ared with the wild-type genotype in those who smoked

more intensely over a shorter period of time in former smokers.

Polymerase chain reaction with confronting two-pair primers (CTPP-PCR) is an effective genotyping method for single nucleotide polymorphisms (SNPs) in aspects of reducing time and costs for analysis. In the present study, the study subject's genotypes of NQO1 by CTPP-PCR method were the same as those genotyped with a RFLP-PCR.

Triplex PCR-RFLP for CYP1A1, GSTM1and GSTT1 polymorphisms has been reported by Bailey et al., [24] compared with PCR-RFLP, PCR-CTPP has the advantage of low cost and rapidity, because it allows genotyping of SNPs without incubation with a restriction enzyme for PCR product digestion. Multiplex PCR-CTPP is applicable; there is no doubt that it is superior to multiplex PCR-RFLP. PCR-CTPP needs less material input and time than PCR-RFLP, even for single polymorphism genotyping. [25]

However, technical problems should be noted for PCR-CTPP. The strength of bands is dependent on the balance in melting temperature of each primer. The balance is also sensitive to annealing temperature of PCR. General speaking, a similar melting temperature for all primers provides the best chance to find an optimal primer set, so primers with a similar melting temperature have to be used. If a suitable primer set cannot be found, this method may not be applicable. This is a common problem to usual PCR with one pair of primers. (14) Kawase et al., (25) reported these conditi - ons after several unsuccessful combinations were tried.

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Global Journal of Medical research: K
Interdisciplinary
Volume 14 Issue 3 Version 1.0 Year 2014
Type: Double Blind Peer Reviewed International Research Journal
Publisher: Global Journals Inc. (USA)
Online ISSN: 2249-4618 & Print ISSN: 0975-5888

Prevalence of Premarital Sexual Practice and Associated Factors among Alamata High School and Preparatory School Adolescents, Northern Ethiopia

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Abstract- Context: Sexual intercourse during adolescence period is unprotected and it has a lot of health risks and physical and psychological hazards. Meanwhile there is no study on the prevalence and associated factors of premarital sexual practice in the study area.

Methods and materials: All regular students attending their school in the year 2013 were included and multi stage sampling method was used. A total of 520 participants were included. Data were collected using self-administered questionnaire.

Aims: The aim of this study is to assess the prevalence of premarital sexual practice and associated factors among Alamata high school and preparatory school adolescents in 2013.

Settings and design: The study was conducted in Alamata, Tigray which is the northern Ethiopia. There is one high school and one preparatory school. The study design was cross sectional.

Keywords: sexual practice, health related problems, alamata, adolescent.

GJMR-K Classification: NLMC Code: WC 140

Strictly as per the compliance and regulations of:

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Statistical analysis used: Data entered using Epl Info version 3.5.1 analyzed using SPSS version 16. Descriptive, bivariate, and multivariate logistic regression analysis were used

Result: A total of 493 participants give complete response. One hundred four (21.1%) of adolescent have had premarital sexual practice. Associated factors were age>18 years (AOR=12, 95%CI=3.97, 36.54), urban resident (preventive) (AOR=0.23, 95%CI=0.07, 0.75), having a positive attitude towards premarital sex (AOR=3.07, 95%CI=1.19, 7.91), having a boy/girlfriend(AOR=3.33, 95%CI=1.39, 7.99), peer pressure (AOR=7.33, 95%CI=2.97, 18.09), and watching sex movies (AOR=7.98, 95%CI=2.55, 24.93).

Conclusion: Prevalence ofpremarital practice was high. Therefore, community and/or school health interventions are needed to reduce the premarital sexual practice among school adolescents to prevent sexually related health problems.

Keywords: sexual practice, health related problems, alamata, adolescent.

Keymessage: lateadolescents, rural resident and having boy/girl friend need special attention in the reduction of premarital sexual practice.

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I. Introduction

According to World Health Organization (WHO) definition adolescent comprises individuals between the age group of 10–19 years old.[1] United Nations International Children's Emergency Fund (UNICEF) categorizes adolescents in to three stages; 10-13 years old as early adolescents, 14-16 years old as middle adolescents, and 17-19 years old as late adolescents.[2]

The World Health Organizations (WHO) has reported that young people aged 15-24 accounted for an estimated 45% of new HIV infections worldwide.[3]

Age at marriage is rising in many African societies, especially among the better-educated and urban segments of the population. At the same time, it is believed that age at menarche is declining, and that premarital adolescent sexual activity is increasing.[4]

According to Ethiopian Demographic and Health Survey (EDHS) 2011 report, among women age 25-49, 29 percent first had sexual intercourse before age 15.[5]

Early sexual debut increases young peoples' risk for infection with HIV and other STIs. Youth who begin early sexual activity are more likely to have high-risk sex or multiple partners and are less likely to use condoms.[6]

In Ethiopia, HIV prevalence in the 15-24 age groups is 8.6%.7 the commonest mode of HIV infection is heterosexual contact accounting 87%. 5, [7]

Studies conducted so far on pre-marital sexual practices and factors related to it in Ethiopia are few in number and there are no current data on this topic in the study area.

II. Materials and Methods

Institutional based cross sectional study design was conducted among high school and preparatory school students in Alamata town, northern Ethiopia in 2013. A total of 520 sample size was calculated using single population proportion formula then the sample was allocated proportionally for all classes. The proc - edure involved of three steps. First, in order to select the study population; half of the sections from each grade

(namely, grade 9, 10, 11, and 12) were selected by using simple random sampling (SRS) Second, a probability proportion to sample size (PPs) was used to select the total number of participants to be included in the study from the randomly selected sections. Third, in order to select the study participants, a systematic random sampling method was used by using roster list or names of the students from the registrar office of both schools in every forth interval.

Data was collected from March to April, 2013 using self administered questionnaire with seven parts namely, Socio-demographic characteristics; Sexual and Reproductive health knowledge and related questions, communication on Sexuality and HIV/AIDS; Attitudes towards pre-marital sex; related to some risk behaviours of Adolescents; Peer Influence and peer behaviour; and sexual behaviour and practices.

Data quality was ensured through pre test was done from other school out of the mail study participants, the questionnaire was translated in to local language-Tigrigna, supervisors and school community were communicated and orientation was given for data collectors and study participants on the purpose of the study.

Data cleaning and entry was performed by using Epl Info version 3.5.1 and analyzed by using SPSS version 16. Descriptive analysis was carried out for the dependent and independent variables. Bivariate analysis was done for the independent variables with the dependent variable to select candidate variables (p - value less than 20%) for multivariate analysis. P value less than 0.05 and 95% confidence interval (CI) for odds ratio was used to judge the significance of association.

Ethical clearance was obtained from Addis Ababa University (AAU), department of nursing and midwifery research committee institutional review board. Permission was granted from officials at different levels in Alamata town through the formal letter obtained from the department's research committee institutional review board.

Administrative and academic staffs were communicated about the study, and they gave their willingness on data collection. Informed consent was obtained from the study subjects after providing the necessary information through the information sheet and the informed consent form which is attached to the front page of the questionnaire and data was collected after getting informed consent from the study participant.

III. Result

a) *Socio demographic characteristics of respondents*

The response rate was 94.8%. From the total respondents, male accounting 264 (53.5%). Among the respondents 472(95.7) were in the age group of 15 to 19 years old. The mean age and standard deviation (SD) for male was 16.7 and 1.48 respectively, and mean age for females was 16.45 and SD of 1.26. (Table 1)

Table 1 : Socio-Demographic Characteristics of Alamata high school and preparatory school adolescent students, Northern Ethiopia, March to April 2013

Variables		Frequency	Percentage
Sex	Male	264	53.5
	Female	229	46.5
Age	10-14 years old	21	4.3
	15-19 years old	472	95.7
Education	Grade 9	227	46.0
	Grade 10	162	32.9
	Grade 11	58	11.8
	Grade 12	46	9.3
Previous residence	Urban	383	77.7
	Rural	110	22.3
Ethnicity	Tigray	452	91.7
	Amhara	41	8.3
Religion	Orthodox	393	79.7
	Muslim	85	17.2
	Protestant	15	3.0
Living condition	With Father and mother	288	58.4
	Mother only or Father only	139	28.2
	Relatives/friends/fiancé	55	11.2
	Alone	11	2.2
Pocket money	Yes	144	29.2
	No	349	70.8

One hundred four (21.1%) of adolescent students participated in this study had had premarital sexual practice. From these, Seventy five (72%) were male and 29 (28%) were females. Age at first sexual intercourse is shown in (fig 1).

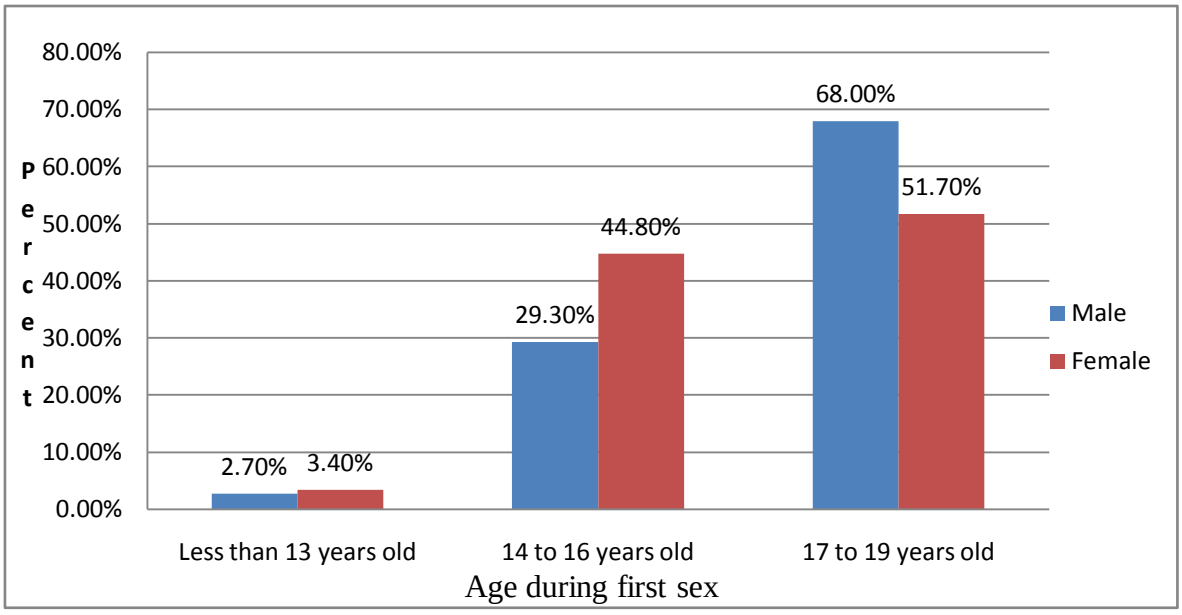

Figure 1 : Age during first sex for male and female among Alamata high school and preparatory school adolescent students, northern Ethiopia, March to April 2013

Sixty six (17.2%) of urban residents and 38(34.5%) of rural residents had had sexual practice before marriage. In addition, 24(77.4%) of respondents who didn't attend church or mosque were engaged in premarital sexual practice, compare to 80(17.3%) of those who attend religious. More than one third of respondents (38.5%) who had sexual practice were from illiterate father, 40(31.5%) of respondents who start sexual practice were from illiterate father, while 8(9.4%) of those who start sexual practice were form a father who have educational level of college and above. Similarly, 47(27.5%) of respondents who start sexual practice were from illiterate mother, while 12(17.1%) of respondents who start sexual practice were from a mother with educational status of college level and above.

Reasons given by the respondents to start sexual practice include fall in love, had desire, peer pressure, raped was drunk and to get money or gift accounting (figure 2).

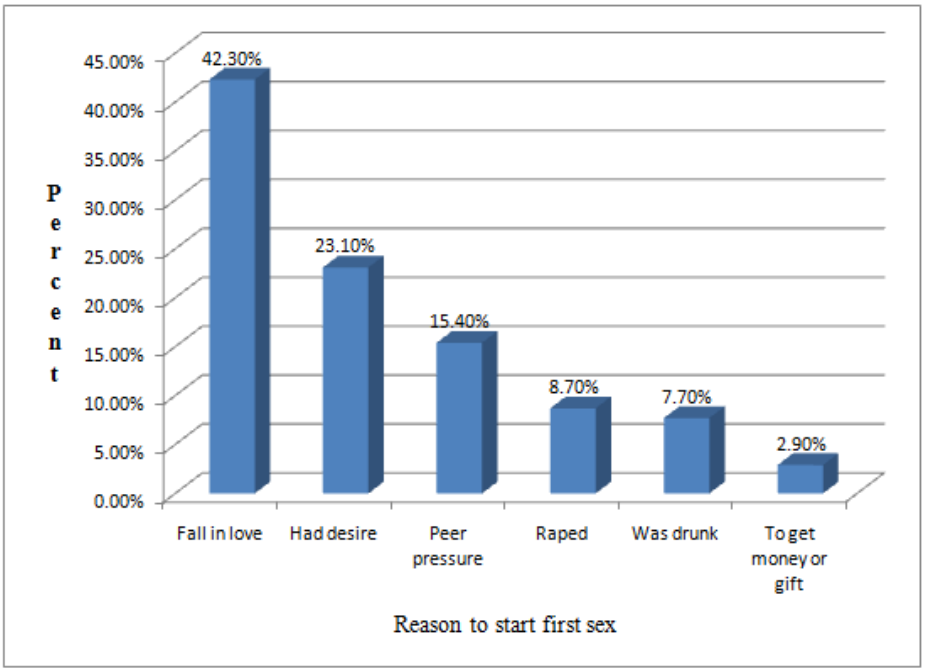

Figure 2 : Reason to start first sex among Alamata high school and preparatory school adolescent students, northern Ethiopia, March to April2013

b) Knowledge and attitude of adolescents about premarital sex

Two third, 318 (64.5%), of study participants had poor knowledge (<mean score (4.1), while 175(35.5%) had good knowledge regarding sexual and reproductive health. Regarding the attitude towards premarital sex, 139(28.2%) of the respondents agree that there is nothing wrong if unmarried boys have sex and 291 (59.0%) of respondents disagree this idea. Regarding the risk behaviors of adolescent's majority, 253(51.3%), of the respondents had never drink alcoholic beverages, while 23.3% drink once or twice a week. Four hundred sixty one (93.5%) had never smoke cigarette in their life, while 21(4.3%) smokes once or twice a week and 3 (0.6%) smokes daily.

More than one third (36.3%) of the respondents said that they have a boy/girlfriend at the time of data collection, while 314(63.7%) don't have a boy/girlfriend (Table 2).

Table 2 : Peer behavior and peer pressure among Alamata high school and preparatory adolescent students, northern Ethiopia, March to April 2013

Variable		Frequency	Percentage
Have boy/girl friend	Yes	179	36.3
	No	314	63.7
Peer pressure	Yes	123	24.9
	No	370	75.1
Male friend sex with prostitute	Yes	69	14
	No	267	54
	I don't know	157	31.8
Best friend had sex	Yes	101	20.5
	No	198	40.2
	I don't know	194	39.4
Place to spend leisure time	Reading books/attend religious ceremony	331	67.1
	Night club	15	3.0
	Others(Watch films with friends, playing with friends, helping family at home)	147	29.8
Watch sex movies	Yes	232	47.1
	No	261	52.9
Frequency of watching sex movies	Once per week	115	49.6
	Twice per week	16	6.9
	Three times and more per week	38	16.4
	Others *	63	27.2

**= every month, twice a month, every 3 months, twice a year, every 1 year*

c) Factors associated with premarital sexual practice

Variables found to have significant association with the premarital sexual practice on bivariate and multivariate analysis are shown in Table 3.

Table 3 : Binary and Multivariate logistic regression indicating factors associated with premarital sexual practice among Alamata high school and preparatory school adolescent students, northern Ethiopia, March to April 2013

Variables		Premarital sex		Crud OR (95% CI)	Adjusted OR(95%CI)	P-value
		Yes	No			
Age	<18 years old	31	313	1	1	
	≥18 years old	73	76	9.7(5.94,15.81)	12.05(3.97,36.53)	0.000*
Previous residence	Urban	66	317	0.39(0.25,0.63)	0.23(0.07,0.75)	0.014*
	Rural	38	72	1	1	
Attitude to prem. sex	Positive	86	167	6.35(3.7,10.96)	3.07(1.19,7.91)	0.020*
	Negative	18	222	1	1	
Use of drugs	Yes	18	5	15.93(5.8,44.1)	8.30(1.17,58.97)	0.034*
	No	85	376	1	1	
Have boy or girl friend	Yes	76	103	7.5(4.6,12.3)	3.33(1.39,7.99)	0.007*
	No	28	286	1	1	
Peer Pressure	Yes	75	48	18.3(10.87,31)	7.33(2.97,18.09)	0.000*
	No	29	341	1	1	
Watch sexual explicit film	Yes	91	141	12.3(6.6,22.8)	7.98(2.55,24.93)	0.000*
	No	13	248	1	1	

**significant association with p<0.05*

IV. Discussion

This study revealed that more than one fifth (21.1%) of respondents were involved in premarital sexual practice. When compared with other studies done on a similar topic, the finding of this study is almost similar with studies done in Nekemt and Injibara.[8, 9] But the finding of this study is relatively higher than the national level of premarital sex. According to BSS, the proportion of premarital sex among in school youths is 16%. This difference could be due to the study population (this study assessed the prevalence of premarital sex among in school students aged 10 to 19 years old, while the national study included only adolescents who are at the age group of 15 to 19 years old). The other reason could be the number of sample used, the current study assessed adolescents found in two schools, while the national survey included a large number sample with diverse population.

According to this study, majority, 75(28.4%) of male respondents and 29(12.7%) of female respondents had had sexual intercourse. This showed that males are highly involved in premarital sexual activity than their counterparts. This could be due to the high expectation of virginity before marriage for females than males and a lesser cultural expectation for males to remain virgin until marriage than females in our society, due to low pare - ntal control for males than females and the differ -ence in the opportunity costs of becoming sexually active (and the subsequent risks of unintended pregnancy).[10, 11] Other similar studies done in eastern Ethiopia, Ambo, Nekemt, Injibara, and Gedeo zone also showed that the proportion of males to engage in premarital sexual practice is relatively higher than females. [8,9,10, 12, 13]A study done among undergraduate university students in Ethiopia also showed that more proportion of male students ever had sex compared to females.[14]Another study done in Jima University students also showed that male students are three times more as likely as their female encounter parts to experience sexual inte - rcourse.[15] Previous studies done in India, Malaysia and China also found that young boys tend to have premarital sexual activity than young girls.[16]

According to the current study, adolescents who are aged 18 and above are more likely to involve in premarital sexual practice than adolescents who are aged below 18 years old. This shows that as the age of adolescents increase, there is higher probability to involve in sexual practice. This result is similar with other studies done in Ambo, Jimma, and Injibara.[9, 12, 15]

Place of residence was another factor found to be associated with premarital sexual practice. According to this study, adolescents who were living in the rural area were more likely to involve in premarital sexual activity than urban residents. This could be because adolescents from rural areas often live away from parental guidance and peoples in urban areas commonly have better sexual and reproductive health access comparing with the rural people.[10, 16, 17] A study conducted in Nekemt town showed that if adolescents migrate from rural areas to urban areas, then it predi - sposes them to unnecessary communications and unsupervised relations which lead them to reckless sex.8A study done in Nigeria regarding internal migr - ation and premarital sexual practice also showed that premarital sexual debut is generally higher among migrants than non-migrants.[17]

In contrast to the current study, a study done in eastern Ethiopia showed that, adolescents from urban families are more likely to engage in pre-marital sex than those from rural area. The reason for this difference could be attributed to a reason that this study involved only 2 schools, while the study in eastern Ethiopia involved 14 schools with diverse population. Another study done in Lesotho showed that norms of the society discouraging sex before marriage have eroded more in urban areas than in rural areas. Hence urban environ - ment provides a conducive environment for experim - enting with sex before marriage. Because of this, never married women living in urban areas are more likely to be sexually experienced than their counterparts residing in rural areas.[18] The difference with the current study could be due to the difference in the socio demographic characteristics and the difference in the study popul - ation (the study in Lesotho included women's aged 15-49 years old while the current study included both male and female in school students whose age is 10 to 19 years old). By considering the above different findings and the reasons given, the association between prem - arital sex and place of residence needs further investigation.

Adolescents with positive attitude towards premarital sexual practice were more likely to engage in premarital sex than those who have negative attitude towards premarital sex. This may be because of the reason that, since the acceptance of sexual practice before marriage encourage adolescents to engage in premarital sexual activity. Other studies done in Jima and Jigjiga also showed that students with moderate and positive attitude towards premarital sexual practice are more likely to engage in sexual activity than their traditional minded students.[15, 19]A study done in Nigeria also showed that personal attitude in favor of delayed sexual debut were associated with lower sexual debut among both males and females.20A study done in Indonesia also showed that, adolescents who accept premarital sexual intercourse have had premarital sexual intercourse than those who do not accept (8.0% and 0.4 respectively).[16]

According to the result of this study, adolescents who use drugs are more likely to involve in premarital sexual practice than non-drug users. This could be because adolescents who abuse drugs are more likely to have poor judgment, which can result in

unplanned and unsafe sex. In addition, most of the adolescents involved in substance use are often also involved in sexual practice.[21] A study done in Maha -rashtra, India on correlates of premarital relationships among unmarried youth also showed that exposure to drugs to be positively associated with rom -antic and sexual relationships for both young women and young men.[22] According to the study done on individual, fam -ilial, friends and contextual predictors of early sexual intercourse, substance use was found to be associated with early sexual intercourse.[21] In addition, a study done regarding sexual initiation, substance use, and sexual behavior and knowledge among vocational students in Northern Thailand also showed that use of substances is associated with initiation of sexual intercourse.[23] Similar findings were also seen in a study done in Malaysia, showing that adolescent sexual intercourse is significantly associated with drug use.[11]

A study done in Indonesia showed that, in school late adolescents who have a boyfriend or girlfriend were more likely to have premarital sexual inte -rcourse than those who do not have a boy/girlfriend.[16] Similar finding was also observed in this study that, adolescents who have a boy/girlfriend are more likely to involve in premarital sex than those who don't have a boy/girlfriend. This could be due to the pressure from their girl/boyfriend to have sexual practice. In Ethiopia, a study done in Jigjiga and Nekemt town also showed that having a boy/girl friend is one factor which dete -rmines to start sexual intercourse.[8,19] Other study done in Malawi also showed that romantic interpersonal relationship was one of the factors that informed sexual decisions of female adolescents.[24]

According to the result of this study, adole -scents who experience pressure from their friends were found more likely to involve in premarital sexual practice than those adolescents who didn't experience peer pressure from their friends. This could be because; peers play important part in deciding personality and behavior among adolescents.[16] In Ethiopia, according the national HIV/AIDS Behavioral Surveillance Survey (BSS), peer pressure was one of the common reasons given for starting sex.[25] Similar result were also observed in studies done in Injibara, Jimma, and Jigjiga showing that students who had peer influence were more likely to have sexual intercourse than students who do not.[9, 15, 19] A study done on premarital sexual debut and its associated factors among in school adolescents in eastern Ethiopia also showed that adolescents who were less influenced by external pressure were more protected against pre-marital sexual debut than their counter parts.[10] Another study done in Nigeria regarding adolescent's susceptibility to peer pressure and how it affects pre-marital sexual practice among adolescents, also showed that susceptibility to peer pressure is signif -icantly associated with premarital sexual practice.[26] Another study regarding the reasons for delaying or engaging in early sexual initiation among adolescents in Nigeria, also showed that peer pressure to be asso -ciated with early sexual initiation.[27]

According to the result of this study, adole -scents who watch sex/pornographic movies were more likely to engage in premarital sexual practice than those who don't. This could be because adolescents who watch pornographic movies may develop unrealistic attitudes about sex, which leads them to engage in sexual practice. A study done regarding the age at sexual initiation and factors associated to it among youths in North East Ethiopia showed that watching pornographic materials at age less than 18 years old was found to be associated with sexual initiation.[28] A study done in Jigjiga also showed that students who watched pornographic/sex movies are more likely to engage in sexual activity.[19] Another study done in Jimma also showed that high and medium level of erotic exposure was more than twice as likely as their peer who had low exposure to erotic materials to be sexual experienced.[15] A study done in Injibara town also showed that students who had not seen sex films were less likely to get in to sexual intercourse than those who had seen such film.[9]The result of this study is also similar with a study done in Asian country about prem -arital sexual intercourse among adolescents. According to the study, pornography viewing was found to be significant factor for premarital sex among boys.[29] In addition a study done in Maharashtra, India, also show -ed that exposure to pornographic films is positively associated with romantic and sexual relationship.[22]

V. Conclusion

A large number of school adolescents were engaged in sexual practice before marriage. Majority of adolescents were males.

The risk factors associated with premarital sexual practice were age (>18 years old), previous residence in rural area, having positive attitude towards premarital sexual practice, use of drugs, having a boy/girlfriend, peer pressure and watching sex movies.

So, community and/or school health intervene -tions are needed to reduce the premarital sexual practice among in school adolescents to prevent sexually related problems by providing due attention for adolescents with above characteristics.

VI. Acknowledgement

We would like to pass our gratitude to Addis Ababa University for the fund. We would also like to thank the data collectors and supervisors who helped us in collecting the data, and for the study participants for their cooperation in filling the data collection instrument.

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Global Journal of Medical research: K
Interdisciplinary
Volume 14 Issue 3 Version 1.0 Year 2014
Type: Double Blind Peer Reviewed International Research Journal
Publisher: Global Journals Inc. (USA)
Online ISSN: 2249-4618 & Print ISSN: 0975-5888

How Much of a Role Birth Asphyxia and Chronic Antenatal Hypoxia Disorders Have in the Genesis of Cerebral Palsy

By Dr. Kulkarni R.S., Dr. Aditya. P. Kulkarni, Dr. Rachana. R. Kulkarni & Dr. Ranjani. R. Kulkarni

Abstract- Objective: Analysis were under taken to determine the role of birth asphyxia and chronic antenatal hypoxia disorders in the genesis of Cerebral palsy, in a prospective study of 31,804 antenatal mothers and 30,080 live births.

Material & Methods: For this large-scale prospective study, proper documentation of all events in the antenatal, natal and postnatal period, a detail, stringent protocol was prepared and distributed to 49 Govt. & Z.P. health institutes. The protocol was filled in for each antenatal mother by the doctor of antenatal clinic and who is attending the delivery. The same was collected back to us by above-mentioned institutes on a fixed date of every month, at the time of monthly review meeting.Thus from 1st Feb 1998 to 31st Jan 2000, a prospective study of 31,804 antenatal mothers were followed up till delivery and 30,080 live births were observed in Sindhudurg district.

Results: 246 children were identified as cerebral palsy in 30,080 live births at the end of 3rd serial examination. Only 33% (82/246) victims of Cerebral palsy had birth asphyxia the presumed cause of their cerebral palsy.

Keywords: cerebral palsy, birth asphyxia, chronic antenatal hypoxic disorders.

GJMR-K Classification: NLMC Code: WS 342

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Large prospective study of 31,804 antenatal mothers followed up till delivery and 30,080 live births observed in Sindhudurg District

Dr. Kulkarni R.S. [α] , Dr. Aditya. P. Kulkarni [σ], Dr. Rachana. R. Kulkarni [ρ] & Dr. Ranjani. R. Kulkarni [ω]

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Results: 246 children were identified as cerebral palsy in 30,080 live births at the end of 3rd serial examination. Only 33% (82/246) victims of Cerebral palsy had birth asphyxia the presumed cause of their cerebral palsy.

Of this 82 cerebral palsy children, 26% (64/246) were of quadriplegic cerebral palsy and 7% (18/246) non-quadriplegic which was attributable to the birth asphyxia. Congenital disorders explained about one third of quadriplegic cerebral palsy. Birth asphyxia was not a significant antecedent of non quadriplegic cerebral palsy.

Conclusion: 33% (82/246) victims of Cerebral palsy had birth asphyxia the presumed cause of their cerebral palsy. There was 20% (48/246) quiet a significant association of cerebral palsy with chronic antenatal hypoxic disorders.

The overall incidence of cerebral palsy for Sindhudurg Dist. amount to 8.1 per thousand live births over a period of 1998 to 2000.

Keywords: cerebral palsy, birth asphyxia, chronic antenatal hypoxic disorders.

I. Introduction

William John Little in 1862, an Orthopaedic Surgeon presented a group of Children with tonal and developmental abnormalities, which he described as spastic rigidity.[1] Many of these children had a history of prolonged labour, preterm delivery. Because of frequency of these perinatal problems, Little postulated that the motor defects resulted directly from difficulties in the birth process. This opinion was widely held for over a century.

Yet there were early critics, chief among them Sigmund Freud, who speculated that, perinatal diffi - culties were the result of pre exisisting abnormalities in the foetus rather than the cause of cerebral palsy.[2]

This study was undertaken to identify and qua - ntitate the major causes of cerebral palsy. The analysis were based on specific disorders that might damage a child's brain.[3] The most widely discussed of these disorders is birth asphyxia, with some people claiming that it is a frequent and others could be misleading because it is possible that such disorders are being missed or that insufficient cases have been analysed to find a correlation between them and cerebral palsy. [4] The first goal of the present study was to determine how much of a role birth asphyxia has in the genesis of cerebral palsy. A second goal was to quantitate the roles of chronic antenatal hypoxia disorders, congenital disorders, hypoglycemia, oxytocin, toxaemia of pregn - ancy,mal presentations and other prenatal factors as causes of cerebral palsy. [5]

II. Material & Methods

38 Primary health centers, 9 Rural hospitals and one Cottage hospital including District hospital are under the technical control of District Civil surgeon. For this large scale prospective study, proper docum - entation of all events in the antenatal, natal and post natal period, a detail, stringent protocol was prepared and distributed to 49 Govt. Rural, Sub District Hospitals & Primary Health Centers. The protocol was filled in for each antenatal mother by the doctors of ante natal clinic and who is attending the delivery. The same was colle - cted back to us by above mentioned institutes on a fixed date of every month, at the time of monthly review meeting at District head quarter with district Civil Surgeon. Thus from 1st Feb 1998 to 31st Jan 2000, a prospective study of 31,804 antenatal mothers were

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followed up till delivery and 30,080 live births were observed in Sindhudurg district. The total number of live births for the above mentioned period was 32366 as per the vital statistics department of District Health Officer, Sindhudurg, thus un accounting the total live births of 2286, which include deliveries in small dispensaries, other nursing homes of outside the districts and home deliveries. All the children born were seen and examined at every six months intervals to identify cerebral palsy by a systematic and uniform record keeping system. The last neurological examination in the study was cond - ucted in February 2002. Data received from above ment -ioned institute enlisted for investigation, which became available for analysis in March 2002. Data for antenatal mothers and intranatal mothers compiled by the respective doctors in the stringent protocols updated, we are fairly confident that the protocol data accurately reflect the Cerebral palsy pattern in Sindhudurg district. 1065 children could not be analysed because the mothers delivered at different hospitals other than above mentioned institutes.

III. Results

Analysis were undertaken in a prospective study of 31,804 ante natal mothers who delivered from 1st Feb 1998 to 31st Jan 2000.. 246 children were identified as cerebral palsy in 30,080 live births at the end of 3rd serial examination.

Table 1 : Various etiological factors and their relative importance / incidence

Sr. No.	Factor	No	Incidence	Relative Risk
1	Prematurity	148/246	60.2%	54.4
2	Low birth weight	136/246	55.3%	52.2
3	Low Apgar score & abnormal foetal heart rate	86/246	34.9%	10.7
4	IUGR on USG	82/246	33%	15.1
5	History of spontaneous abortion & stillbirth	68/246	27.06%	5.7
6	Toxaemia of pregnancy	44/246	17.9%	8.1
7	Forceps application	42/246	17%	42.2
8	Muconium stained liquor	36/246	14.7%	1.2
9	Malpresentation	34/246	13.9%	8.6
10	Oxytocin drip during labour	26/246	10.7%	2.5
11	Unusually long or short interval between pregnancy	26/246	10.6 %	1.9
12	Caesarian section	16/246	6.5%	0.78
13	History of taking thyroid / oestrogen hormones	16/246	6.5%	43.5
14	Vaccum application	12/246	4.9%	29.6
15	Post maturity	12/246	4.9%	1.6
16	Bleeding during 1st , 2nd, 3rd, trimester of pregnancy	8/246	3.3%	1.3

The following positive antenatal, intranatal findings noticed are suggestive of quite a significant association of cerebral palsy with chronic antenatal hypoxia disorders.

60.2% (148/246) were born prematurely before 32 weeks of pregnancy. 55.3% (136/246) were low birth weight babies (below 2500 grams). Low Apgar scores & abnormal foetal heart rate during labour were present in 34.9% (86/246). Evidence of IUGR on USG was diag - nosed in 33% (82/246). History of spontaneous abortion and still births were detected in 27.6% (68/246). Toxa - emia of pregnancy was noted in 17.9% (44/246) ante natal mothers. Forceps were applied during deliveries in 17% (42/246). Muconium stained liquor during labour was seen in 14.7% (36/246). Mal presentations were seen in 13.9% (34/246). Oxytocin drip was started during labour in 10.6% (26/246). An unusually long or short interval between the pregnancy was seen in ante natal mothers cerebral palsy children 10.6% (26/246). Caesarean section was performed in 6.5% (16/246) in pregnant women. Ante natal mothers with history of taking thyroid hormones and oestrogen in 6.5% (16/246) were noted. Vacuum was applied during delivery in 4.9% (12/246) ante natal mothers. Post maturity was visu - alised in 4.9% (12/246). Bleeding during 1st 2nd & 3rd trimester of pregnancy was seen in 3.3% (8/246).

Thus 33% (82/246) victims of Cerebral palsy had birth asphyxia the presumed cause of their cerebral palsy. Of this 26% (64/246) were cases of quadriplegic cerebral palsy and 7% (18/246) non quadriplegic, which was attributable to the birth asphyxia. There was quiet a significant association of cerebral palsy with chronic antenatal hypoxic disorders. Congenital disorders explained about one third of quadriplegic cerebral palsy. Birth asphyxia was not a significant antecedent of non quadriplegic cerebral palsy.

Finally the findings of the present study under score the importance of making accurate measure - ements and observations on neonates to avoid mistakes attributing non asphyxial cerebral palsy to birth asp - hyxia. The overall incidence of cerebral palsy for Sindhudurg Dist. amount to 8.1 per thousand live births over a period of 1998 to 2000.

IV. Discussion

Most studies that have attempted to determine if birth asphyxia is a cause of cerebral palsy, have used low Apgar scores and foetal distress to identify asp -

hyxia. Low Apgar scores and foetal distress are often non hypoxic in origin, so their use as indicators of birth asphyxia could misattribute some non asphyxial cer - ebral palsy to asphyxia.[6] We explored this possibility by seeing how many victims of cerebral palsy who had low Apgar scores had a non asphyxial disorder as the basis for their cerebral palsy.

During the past two decades, dramatic changes in obstetrical and perinatal care have included the increasing availability of foetal heart monitoring and foetal ultrasonography, the establishment of neonatal intensive care units, and the implementation of policies to encourage the regionalization of care and the tran - sport of mothers carrying high-risk foetuses before delivery. If the occurrence of cerebral palsy reflected sub optimal obstetrical care, [6] then its prevalence would be expected to decline in response to these remarkable improvements in care, but it has not done so.[8]

In an attempt to evaluate the relative contribution of all pregnancy-related factors, some epidemiologists have created analytic models that evaluate later events (for example, those occurring during the delivery)[9] in the light of earlier events (characteristics of the mother before pregnancy, first-trimester events, and so on).[10] ,in the victims of cerebral palsy, characteristic consequences of birth asphyxia were more often the result of non-asphyxial disorders.[11] These included muconium in the amniotic fluid, low 10 minute Apgar scores.

Another perspective is gained by looking at the relative risks of various risk factors for cerebral palsy. Birth asphyxia had the highest relative risk for quadriplegic cerebral palsy. However, the low frequency of birth asphyxia in the population as a whole (82 of 30804) gave birth asphyxia a much smaller role as a cause of quadriplegic cerebral palsy.

Difference in distribution of factors related to cerebral palsy is highly significant Since these factors are not mutually exclusive i.e. same case of cerebral palsy can have more than one factor hence chi square test won't make any sense really.

Factor / Disease	cerebral palsy +	cerebral palsy --	
LBW +	a 136	b X	a + b
Normal born wt	c 110	d Y	c + d
	a + c 246	b+d 29834	A+b+c+d 30080

$$\text{Relative risk} = \frac{\text{Incidence of disease in exposed group}}{\text{Incidence of disease in nonexposed group}} = \frac{\frac{a}{a+b}}{\frac{c}{c+d}}$$

a) Interpretation of Relative risk

If RR = 1, Then it means no risk

If RR = > 1, means more risk

In this study the highest relative risk is for Prematurity. The risk of cerebral palsy is 54.4 times more in premature babies than those born with normal birth weight i.e. premature babies are 54.4 times at an added risk of cerebral palsy than normal babies.

The second important risk factors in descending order are low birth weight (RR = 52.2), history of taking thyroid / oestrgen hormones (RR = 43.5) and Foreceps application (RR=42.2)

In this study of all 16 factors only for caesarian section value of Relative risk is < 1 i.e. 0.78 (it indicates protective effiect) i.e. Babies delivered by caesarian section have less risk of cerebral palsy than other babies.

Table 2

Sr. No.	O	E	$(O-F)^2/E$
1	136	49.5	151.1
2	148	49.5	196.0
3	26	49.5	11.1
4	68	49.5	6.9
5	34	49.5	4.8
6	12	49.5	28.4
7	86	49.5	26.9
8	36	49.5	3.6
9	16	49.5	22.6
10	8	49.5	34.7
11	44	49.5	0.6

12	82	49.5	21.3
13	42	49.5	1.1
14	12	49.5	28.4
15	16	49.5	22.6
16	26	49.5	11.1
			571.2

$$EX = 792 \times = \frac{E}{X} = 49.5\ X2 = 571.2,\ df = 15\ p<0.001$$

b) Difference is highly significant statistically

A child whose mother has long intervals between menses appears to be at increased risk for cerebral palsy.[12] The risk is increased if there has been an unusually short interval (less than three months) or an unusually long interval (more than three years) since the previous pregnancy. [13] In addition, mothers of children with cerebral palsy are more likely than other mothers to have a history of spontaneous abortion and stillbirth. These findings indicate that maternal menstrual and obstetrical factors convey information about the risk of cerebral palsy.

Twins are more likely than singletons to have antenatal peri ventricular leukomalacia[14] and cerebral palsy. [15] Some of the increased risk of cerebral palsy among twins probably results from their gestational age and intrauterine growth retardation. In one study, an increase in the cesarean-section rate in the delivery of twins was not associated with a reduction in the prev - alence of cerebral palsy.[16]

The greater concordance for cerebral palsy among monozygotic than dizygotic twins also suggests a genetic basis, but it is compatible with placental prob - lems that are unique to monozygotic twins as well.

Mothers known to have been hyperthyroid or who were prescribed thyroid hormones or estrogen in pregnancy have been found to be at increased risk of giving birth to a child in whom cerebral palsy later dev - elops.

Non-vertex and face presentations of the foetus are associated with an increased risk of cerebral palsy. [17] One interpretation of this fact is that an abnormal presentation does not cause cerebral palsy, but rather may be a marker of preexisting difficulties. According to this hypothesis, foetuses with hypotonia and other abnormalities that will later be manifested as cerebral palsy are less able than others to move into a vertex position.

The rate of cerebral palsy is 25 to 31 times higher among infants who weigh less than 1500 g at birth than among full- sized newborns.[18] Babies whose birth weight is less than 2500 g account for about one third of all babies who later have signs of cerebral palsy. [19]

As a generalization, the lower the birth weight and the gestational age, the higher the risk of cerebral palsy [20] and peri ventricular leukomalacia. Thus it should not be surprising that a number of low birth weight and early gestational age children are associated with peri ventricular leukomalacia, even among babies born prematurely.[21]

Nelson and Ellen berg wrote in 1986 "Of the . . . mother-infant pairs in the 5 percent with the highest risk (for cerebral palsy) only 208 percent produced a child with cerebral palsy, the false positive rate was thus 97 percent." Epidemiological studies published since then have not provided any reasons to change the impression that our ability to identify modifiable presumed causes of cerebral palsy is limited.

The burden imposed by cerebral palsy on society has not abated despite recent advances in medical care. Indeed, the increased survival of preterm newborns at risk for the disease has resulted in an increased number of children with cerebral palsy, mainly of the spastic diplegic variety. [22]

Table 3 : Incidence of cerebral palsy compared with the findings of published literatuve

Sr. No.	Author	Year	Country	Incidence
1	Fiona J. Stanley	1967 to 1985	Western Australia	2.5 to 5/1000 live births
2	Rosen MG	1992	USA	1 to 6 /1000 live births
3	Sofia franco		Kentucky USA	2.1 /1000 live births
4	Peggy S. Eicher	1993	Pennsylvania	2 /1000 live births
5	Mercer Rang	1993	Canada	5 /1000 live births
6	Kulkarni R.S.	1998 - 2000	Maharastra, India	8.1 /1000 live births

Thus, efforts to prevent cerebral palsy will require a focus on factors and events during pregnancy including those that predispose the mother and foetus to preterm delivery and low birth weight.

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Global Journal of Medical research: K
Interdisciplinary
Volume 14 Issue 3 Version 1.0 Year 2014
Type: Double Blind Peer Reviewed International Research Journal
Publisher: Global Journals Inc. (USA)
Online ISSN: 2249-4618 & Print ISSN: 0975-5888

Potential Health Benefits and Adverse Effects Associated with Phytate in Foods: A Review

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Abstract- Phytate (myo-inositol (1,2,3,4,5,6) hexakis - phosphate), a naturally compound formed during maturation of plant seeds and grains is a common constituent of plant-derived foods. This paper is aimed to review the scientific information concerning the potential health benefits and adverse effects associated with phytate in foods. The adverse health effects of phytate in the diet is its effect on mineral uptake. Minerals of concern in this regard would include Zn2+, Fe2+/3+, Ca2+, Mg2+, Mn2+, and Cu2+. Especially zinc and iron deficiencies were reported as a consequence of high phytate intakes. In addition, a the adverse effect on the nutritional value of protein by dietary phytate is discussed. Consumption of phytate, however, seems not to have only adverse health effects but also potential benefits on human health. Dietary phytate was reported to prevent kidney stone formation, protect against diabetes mellitus, caries, atherosclerosis and coronary heart disease as well as against a variety of cancers.

Keywords: *antinutrient, phytate, health benefits, health effects, human nutrition.*

GJMR-K Classification: *NLMC Code: QU 50*

Strictly as per the compliance and regulations of:

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I. Introduction

Phytate (is also known as Inositol hexakis - phosphate (InsP6)) is the salt form of phytic acid, are found in plants, animals and soil. It is primarily present as a salt of the mono- and divalent cations K+, Mg2+, and Ca2+ and accumulates in the seeds during the ripening period. Phytate is regarded as the primary storage form of both phosphate and inositol in plant seeds and grains [1]. In addition, phytate has been suggested to serve as a store of cations, of high energy phosphoryl groups, and, by chelating free iron, as a potent natural anti-oxidant [2,3].

Phytate is ubiquitous among plant seeds and grains, comprising 0.5 to 5 percent (w/w) [1]. The phosphorus bound to phytate is not typically bio-available to any animal that is non-ruminant. Ruminant animals, such as cows and sheep, chew, swallow, and then regurgitate their food. This regurgitated food is known as cud and is chewed a second time. Due to an enzyme located in their first stomach chamber, the rumen, these animals are able to separate, and process the phosphorus in phytates. Humans and other non-ruminant animals are unable to do so [4].

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Phytate works in a broad pH-region as a highly negatively charged ion, and therefore its presence in the diet has a negative impact on the bioavailability of dival - ent, and trivalent mineral ions such as Zn2+, Fe2+/3+, Ca2+, Mg2+, Mn2+, and Cu2+ [6]. Whe -ther or not high levels of consumption of phytate-containing foods will result in mineral deficiency will depend on what else is being consumed. In areas of the world where cereal proteins are a major and pred -ominant dietary factor, the associated phytate intake is a cause for concern [27].

Besides, phytate has also been reported to form complexes with proteins at both low, and high pH values. These complex formations alter the protein structure, which may result in decreased protein solub - ility, enzymatic activity, and proteolytic digestibility. The phytate degrading enzyme, phytase, is in vogue for degradating phytate during food processing, and in the gastrointestinal tract. The major concern about the presence of phytate in the diet is its negative effect on mineral uptake [28]. Phytate markedly decrease Ca bioavailability, and the Ca:Phy molar ratio has been proposed as an indicator of Ca bioavailability. The critical molar ratio of Ca: Phy is reported to be 6:1 [29]. In human studies, Phy:Zn molar ratios of 15:1 have been associated with reduced zinc bioavailability, and the molar ratio [Ca][Phy]/[Zn] is a better predictor of zinc availability, because calcium exacerbates phytate's effect on zinc absorption, and if the values were greater than 0.5 mol/kg, there would be interference with the availability of zinc [30].

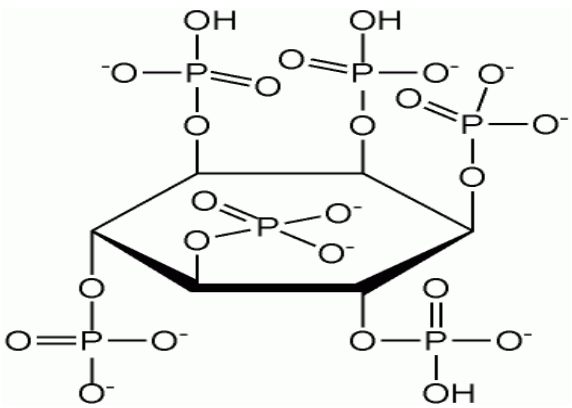

Figure 1 : Structure of Phytate (Insp6), empirical formula=C6P6O24H18

At the same time, phytate may have beneficial roles as an Antioxidant, and Anticarcinogen [31]. The outcome of surveillance of populations consuming veg - etarian-type diets has shown lower incidence of Cancer, which suggests that phytate has an Anticarcinogen effect [32]. Dietary phytate may have health benefits for Diabetes patients because it lowers the blood glucose response by reducing the rate of starch digestion and slowing gastric emptying. Likewise, phytate has also been shown to regulate Insulin secretion [33]. It is believed that phytate reduces Blood clots, Cholesterol, and Triglycerides, and thus prevents Heart diseases. It is also suggested that it prevents renal stone develop - pment. It is used as a complexing agent for rem -oval of traces of heavy metal ions [34].

Depending on the amount of plant-derived foods in the diet, and the grade of food processing, the daily intake of phytate can be as high as 4500 mg. On average, daily intake of phytate was estimated to be 2000–2600 mg for vegetarian diets as well as diets of inhabitants of rural areas in developing countries, and 150–1400 mg for mixed diets [35, 37. 38]. Among the cooking treatments boiling appeared effective to reduce the phytate level, which could reduce as high as 20% of phytate [36, 39]. However, the updated information on health benefits and adverse effects of phytate in foods is scant. Therefore, the objective of this review is to assess updated scientific information of the potential health benefits and adverse effects associated with phytate in foods.

II. Adverse Health Effects of Phytate

The major concern about the presence of phytate in the diet is its negative effect on mineral uptake. Minerals of concern in this regard would include Zn2+, Fe2+/3+, Ca2+, Mg2+, Mn2+, and Cu2+ [13,14], but also a negative effect on the nutritional value of protein [5,7].

a) Effect on mineral uptake

Phytate forms complexes with numerous divalent and trivalent metal cations. Stability and solu - bility of the metal cationphytate complexes depends on the individual cation, the pH-value, the phytate:cation molar ratio, and the presence of other compounds in the solution [15]. Phytate has six reactive phosphate groups and meets the criterion of a chelating agent. In fact, a cation can bind to one or more phosphate group of a single phytate molecule or bridge two or more phytate molecules [3, 40]. Most phytates tend to be more soluble at lower compared to higher pH-values [16]. Solubility of phytates increase at pH-values lower than 5.5-6.0 with Ca2+, 7.2-8.0 with Mg2+ and 4.3-4.5 with Zn2+ as the counter ion. In contrast, ferric phytate is insoluble at pH values in the range of 1.0 to 3.5 at equimolar Fe3+ : phytate ratios and solubility increases above pH 4 [17]. Another important fact is the synergistic effect of secondary cations, among which Ca2+ has been most prominently mentioned [18, 41]. Two cations may, when present simultaneously, act jointly to increase the quantity of phytate precipitation. For example, Ca2+ enhanced the incorporation or adsorption of Zn2+ into phytate by formation of a calcium-zinc phytate. The effect of Ca2+ on the amount of Zn2+ co-precipitating with phytate is dependent on the Zn2+ : phytate molar ratio. For high Zn2+ : phytate molar ratios, Ca2+ displaces Zn2+ from phytate bin - ding sites and increases its solubility. The amount of free Zn2+ is directly proportional to the Ca2+-conce - ntration. For low Zn2+: phytatemolar ratios, Ca2+ potentiate the precipitation of Zn2+ as phytate. Thus, higher levels of Ca2+ result in a more extensive precipitation of the mixed phytates. Mg2+ also has been shown in vitro to potentiate the precipitation of Zn2+ in the presence of phytate, however, Mg2+ has been found to exert a less pronounced effect on Zn2+- solubility than Ca2+ [18, 42].

The knowledge about the interaction of partially phosphorylated myo-inositol phosphates with different cations is limited. Recent studies have shown that myo-inositol pentakis-, tetrakis- and trisphosphates have a lower capacity to bind cations at pH-values ranging from 5.0 to 7.0 [19]. The capacity to bind cations was found to be a function of the number of phosphate groups on the myo-inositol ring. The cation-myo-inositol phosphate complexes are more soluble as the number of phos - phate groups decreases. There is also some evid -ence for weaker complexes when phosphate groups are removed from phytate. In addition, the binding affinity of cations to myo-inositol phosphates has been shown to be affected by the distribution of the phosphate residues on the myo-inositol ring.

The formation of insoluble metal cation-phytate complexes at physiological pH-values is regarded as the major reason for a poor mineral availability, because these complexes are essentially non-absorbable from the gastrointestinal tract. Most studies have shown an inverse relationship between phytate content and mineral availability, although there are great differences in the behaviour of individual minerals. Zn2+ was reported to be the essential mineral most adversely affected by phytate [13,14]. Zn2+ deficiency in humans was first reported in 1963 in Egyptian boys whose diets consisted mainly of bread and beans [20, 43]. These patients, who were characterised by dwarfism and hy - pogonadism, showed a response to dietary Zn2+ supplementation. It became accepted that the presence of phytate in plant-based foods is an important factor in the reduction of Zn2+ absorption.

Phytate affects Zn2+ absorption in a dose-dependent manner. There is, however, some lack of agreement among studies, particularly with respect to specific foods and their individual components. In addition, phytate was shown not only to depress the

availability of dietary Zn2+, but also to affect Zn2+ homeostasis negatively [15]. A great deal of controversy exists regarding the effect of phytate on the availability of dietary iron [14, 21]. Much of this controversy may be due to the low absorption of iron in general, the presence of different iron-phytates with different solu - bility, and the existence of two types of food iron, heme and nonheme iron.

Heme iron is better absorbed and its absorption is little affected by dietary factors; nonheme iron, however, is less easily absorbed, and its absorption is affected by other dietary factors. Since many human studies indicate that phytate has a very strong inhibitory effect on iron absorption, it is well accepted today, that phytate appears to be the major but not the only contributor to the reduction in iron availability in man [22, 44]. Human studies also indicated that phytate inhibits Ca2+ absorption, but the effect of phytate on Ca2+ availability seems to be less pronounced compared to that on the availability of iron and particularly Zn2+ [7, 14]. This may be due to the relatively high Ca2+ content of plant-based foods, the capability of the bacterial flora in the colon to dephosphorylate phytate and the fact, that Ca2+ could be absorbed from the colon [23]. Relatively few studies have dealt with the effects of phytate on dietary Cu2+, Mn2+ and Mg2+ utilisation. Phytate has been shown to decrease their bioavailability in in vivo studies, but it appears that the effect of phytate on Cu2+, Mn2+ and Mg2+ availability is less marked than those for some other essential elements [13,14].

The fact that phytate phosphorus is poorly available to single stomached living beings including man was already demonstrated [24, 25]. Phosphorus is absorbed as ortho-phosphate and therefore the utilis - ation of phytate-phosphorus by single-stomached living beings will largely depend on their capability to dephosphorylate phytate. It was already shown, that the human small intestine has only a very limited capability to hydrolyse phytate [26] due to the lack of endogenous phytate-degrading enzymes (phytases) and the limited microbial population in the upper part of the digestive tract.

b) Effect on protein digestibility

Phytate interactions with proteins are pH-dependent [5, 7]. At pH-values below the isoelectric point of the protein, the anionic phosphate groups of phytate bind strongly to the cationic groups of the protein to form insoluble complexes that dissolve only below pH 3.5. The α-NH2 terminal group, the ε-NH2 of lysine, the imidazole group of histidine and guanidyl group of arginine have been implicated as protein bin - ding sites for phytate at low pH-values. These low pH proteinphytate complexes are disrupted by the comp - etitive action of multivalent cations. Above the isoelectric point of the protein, both protein and phytate have a neg -ative charge, but in the presence of multivalent cations, however, soluble protein-cation-phytate complexes occur. The major protein binding site for the ternary complex appears to be the nonprotonated imidazole group of histidine, but the ionized carboxyl group of the protein are also suggested sites. These complexes may be disrupted by high ionic strength, high (pH> 10), and high concentrations of the chelating agents.

Phytate is known to form complexes with proteins at both acidic and alkaline pH [5]. This inte - raction may effect changes in protein structure that can decrease enzymatic activity, protein solubility and proteolytic digestibility. However, the significance of protein-phytate complexes in nutrition is still under scrutiny. Strong evidence exists that phytate-protein interactions negatively affect protein digestibility in vitro and the extent of this effect depends on the protein source [5]. A negative effect of phytate on the nutritive value of protein, however, was not clearly confirmed in studies with simple-stomached animals [7, 45]. While some have suggested phytate does not affect protein digestibility, others have found an improvement in amino acid availability with decreasing levels of phytate. This difference may be at least partly due to the use of different protein sources. Of nutritional significance might be also the inhibition of digestive enzymes such as α-amylase [46,47], lipase [48] or proteinases [49,51], such as pepsin, trypsin and chymotrypsin, by phytate as shown in in vitro studies. The inhibitory effect increases with the number of phosphate residues per myo-inositol molecule and the myo-inositol phosphate concentration. This inhibition may be due to the non-specific nature of phytateprotein.

interactions, the chelation of calcium ions which are essential for the activity of trypsin and α-amylase, or the interaction with the substrates of these enzymes. The inhibition of proteases may be partly responsible for the reduced protein digestibility. Phytate has also been considered to inhibit α-amylase in vivo as indicated by a negative relationship between phytate intake and blood glucose response [50, 52].

III. Beneficial Health Effects of Phyate

In the view of the above results, the evidence seems overwhelming that high intakes of phytate can have adverse effects on mineral uptake in humans. In the last years, however, some novel metabolic effects of phytate or some of its degradation products have been recognised. Dietary phytate was reported to prevent kidney stone formation [8], protect against diabetes mellitus [9], caries [10], atherosclerosis and coronary heart disease [11] as well as against a variety of cancers [12]. The levels of phytate and its dephosphorylation products in urine, plasma and other biological fluids are fluctuating with ingestion or deprivation of phytate in the human diet [53]. Therefore, the reduction in phytate intake in developed compared to developing countries

might be one factor responsible for the increase in diseases typical for Western societies such as diabetes mellitus, renal lithiasis, cancer, atherosclerosis and coronary heart diseases. It was suggested that phytate exerts the beneficial effects in the gastrointestinal tract and other target tissues through its chelating ability, but additional mechanisms have also been discussed. Moreover, the potential beneficial effects of phytate in the prevention of severe poisoning should be consi - dered.

One to two percent calcium phytate in the diet has been found to protect against dietary Pb2+ in experimental animals and in human volunteers [54]. Furthermore, calcium phytate was capable of lowering blood Pb2+ levels [7, 55]. Thus, phytate seems to be a helpful means to counteract acute oral Pb2+ toxicity. The effect of calcium phytate on acute Cd2+ toxicity is still discussed controversially, but the majority of studies point to an improved Cd2+ absorption in the presence of phytate [56,57]. This may result in a Cd2+ accum - ulation in liver and kidney.

Diabetes mellitus is one of the most common nutrition-dependent diseases in Western society. It may be caused by hyper-caloric diets with high percentage of quickly available carbohydrates. Foods that result in low blood glucose response have been shown to have great nutritional significance in the prevention and management of diabetes mellitus. In this regard phytate-rich foods are of interest, since a negative relationship between phytate intake and blood glucose response was reported [9,52]. For example, phytateenriched unleavened bread based on white flour reduced the in vitro starch digestibility besides flattening the glycemic response in five healthy volunteers in comparison with bread without phytate addition [52]. The in vitro reduction of starch digestion was positively correlated with the myo-inositol phosphate concentration and negatively with the number of phosphate groups on the myo-inositol ring. It has to be noted, that there are also studies which have not found an inhibition of α-amylase and starch digestion by phytate.

a) Phytate and Coronary Heart Disease

Heart disease is a leading cause of death in Western countries, yet it is low in Japan and developing countries. Elevated plasma cholesterol or more speci - fically, elevated Low Density Lipoprotein chole -sterol concentrations have been shown to be one of the risk factors. It has been proposed that dietary fibre or more specifically phytate, as a component of fibre, may influ - ence the aetiology of heart disease [58]. Animal studies have demonstrated that dietary phytate supplementation resulted in significantly lowered serum cholesterol and triglyceride levels [11]. This effect was accompanied by decrease in serum zinc level and in zinc-copper ratio. Thus, the hypothesis was put forward that coronary heart disease is predominantly a disease of imbalance in regard to zinc and copper metabolism [59]. The hypothesis is also based on the production of hyperch - olesterolemia, which is a major factor in the aetiology of coronary heart disease, in rats fed a diet with a high ratio of zinc and copper [60]. It was thought that excess zinc in the diets resulted in decreased copper uptake from the small intestine, since both minerals compete for common mucosal carrier systems. As phytate preferentially binds zinc rather than copper [61], it was presumed that phytate exerts its effect probably by decreasing zinc without affecting copper absorption. It should be pointed out that the support for the preventive role of phytate in heart disease is based only on a few animal and in vitro studies. Results from human studies are still lacking.

b) Phytate and Renal Lithiasis

The increase of renal stone incidence in northern Europe, North America, and Japan has been reported to be coincident with the industrial develop - pment of these countries, making dietary intake suspect. Epidemiological investigations found that there were substantial differences in renal stone incidences between white and black residents of South Africa [62]. The major dietary difference is that, compared to the white population, blacks consumed large amounts of foods containing high levels of fibre and phytate. Furthe -rmore, a high phytate diet has been used effect -tively to treat hypercalciuria and renal stone formation in humans [7, 63]. In recent years, research on phytate as a potent inhibitor of renal stone formation has been intensified [8, 64,65]. By comparing a group of active calcium oxalate stone formers with healthy people it was demonstrated that urinary phytate was significantly lower for stone formers [8]. Therefore, in vitro and in vivo experiments as well as clinical studies clearly demon - strate that phytate plays an important role in preventing the formation of calcium oxalate and calcium phosphate crystals, which function as nuclei for kidney stone devel -opment. Because excretion of low phytate amounts in the urine was shown to be an important risk factor in the development of renal calculi and urinary excretion of phytate decreased significantly after intake of a phytate-free diet [64], the importance of dietary phytate in maintaining adequate urinary levels to permit effective crystallization inhibition of calcium salts and conseq - uently preventing renal stone development was demo - nstrated.

c) Phytate and Cancer

The frequency of colonic cancer varies widely among human populations. It is a major cause of mor - bidity and mortality in Western society. The incidence of cancer, especially large intestinal cancer has been associated principally with dietary fat intake and is inversely related to the intake of dietary fibre. It was further suggested that the apparent relationship bet - ween fibre intake and rate of colonic cancer might arise

from the fact that many fibre-rich foods contain large amounts of phytate and that this latter might be the critical protective element, since an inverse correlation between colon cancer and the intake of phytate-rich fibre foods, but not phytatepoor fibre foods has been shown [66]. A high phytate intake may also be an important factor in reducing the breast and prostate cancer mortality in man [12]. Both in vivo and in vitro experiments have shown striking anticancer effects of phytate. It was demonstrated that phytate is a broad-spectrum antineoplastic agent, affecting different cells and tissue systems [12]. Phytate inhibited the growth of human cell lines such as leukaemic haematopoietic K-562 cell line [67,68], colon cancer HT-29 cell line [69], breast cancer cell lines [70], cervical cancer cell lines [71], prostate cancer cell lines [72,74], HepG2 haep -atoma cell line [75], mesenchymal tumour cells [76], murine fibrosarcoma tumour cells [76], and rhabdom -yosarcoma cells [77] in a dose- and time-dependent manner. However, cells from different origin have diff -erent sensitivity to phytate, suggesting that phytate may affect different cell types through different mechanisms of action. It was also demonstrated, that phytate has the portential to induce differentiation and maturation of malignant cells, which often results in reversion to the normal phenotype [68]. Phytate was further shown to increase differentiation of human colon carcinoma HT-29 cells [69,78], prostate cancer cells [72, 73], breast cancer cells [70], and rhabdo -myosarcoma cells [77]. The effectiveness of phytate as a cancer preventive agent was also shown in colon cancer induced in rats and mice. Phytate was effective in a dose-dependent manner given either before or after carcinogen admin -istration.

The phytate-treated animals demonstrated a significantly lower tumour number and size. Studies using other experimental models showed that the antineoplastic properties of phytate were not restricted to the colon. Phytate significantly reduced experimental mammary carcinoma [79,80, 83], skin papillomas [84], tumour size of metastatic fibrosarcoma and exper -imental lung metastases [76], growth of rhabdomyo -sarcoma cells [77], and regression of pre-existing liver cancers [75,85]. In addition synergistic cancer inhibition by phytate when combined with inositol was demo -nstrated in several cancers in experimental animals [76,81,82,86]. The in vivo experiments were performed either by adding phytate to the diet or by giving phytate via drinking water. Comparable of even stronger tumour inhibition was obtained with much lower concentrations of phytate when it was given in drinking water.

d) Mechanism of action

The mechanisms involved in the anticancer activity of phytate are not fully understood. It was sugg -ested that phytate exerts the beneficial effects through its chelating ability, but additional mechanisms have also been discussed. Because several myo-inositol phosphates, including phytate, are present as intra -cellular molecules and because the second messenger D-myo-inositol (1,4,5) trisphosphate is brin -ging about a range of cellular functions including cell proliferation via mobilising intracellular Ca2+ [87], phytate was propo-sed to exert its anticancer effect by affecting cell signalling mechanisms in mammalian cells [68]. About 35 of the 63 possible myo-inositol phos -phate isomers were identified in different types of cells [87]. Depending on cell type, that is different receptors, phosphatases, and kinases, myo-inositol phosphates were linked with different physiological effects, such as basic cell func -tions like secretion and contraction as well as functions like cell division, cell differentiation and cell death. Therefore, practically every myo-inositol phos -phate isomer extracellularly present and may have a metabolic effect by activating receptors, by being meta -bolised by phosphatases and kinases or by acting as inhibitors of these intracellular proteins after being internalised by cells. An effect of extracellular phytate on the conce -ntration of several in- tracellular myo-inositol phosphate esters has already been demonstrated in human erythroleukemia cells [68]. Furthermore, it has been recently reported that highly negatively charged myo-inositol polyphosphates can cross the plasma mem -brane and be internalised by cells. Myo-inositol hexakis -phosphate was shown to enter HeLa cells followed by an intracellular dephosphorylation to partially phosph -orylated myo-inositol phosphates [71], whereas myo-inositol (1,3,4,5,6) pentakisphosphate showed a quite slow turnover after internalisation by SKOV-3 cells [88]. It was suggested that the anticancer activity of phytate is actually due to its dephosp -horylation to lower forms. Myo-inositol (1,3,4,5, 6) pentakisphosphate inhibits specifically phosphatidylinositol 3-kinase, the enzyme catalysing the phosphorylation of inositol phospholipids at the D3 position to generate 3'-phosphorylated phosphoinositides [89], which act by recruiting specific signalling proteins to the plasma membrane [90]. Activation of phospha -tidylinositol 3-kinase is a crucial step in some events leading to angiogenesis, the form -ation of a mature vasculature from a primitive vascular network [90, 91]. Angiogenesis is involved in pathologies such as arteriosclerosis and tumour growth.

The observed anticancer effects of phytate could be mediated through several other mechanisms. Besides affecting tumour cells, phytate can act on a host by restoring its immune system. Phytate augments natural killer cell activity in vitro and normalises the carcinogen-induced depression of natural killer cell activity in vivo [7, 92]. The anti-oxidant role of phytate is known and widely accepted. The 1,2,3-trisphosphate grouping in phytate has a conformation that uniquely provides a specific interaction with iron to completely inhibit its capability to catalyse hydroxyl radical form -ation from the Fenton reaction [93]. Chelation of iron to the 1, 2, 3-trisphosphate grouping may also reduce the

likelihood for ironcatalysed lipid peroxidation [94]. It is as yet uncertain whether physiological intakes of phytate can significantly improve the anti-oxidant status in man. The anticancer action of phytate may be further related to mineral binding ability or other positively charged compounds. By complexing Zn2+ and /or Mg2+, phy - tate can affect activity of enzymes essential for DNA synthesis. Due to inhibition of starch digestion in the small intestine, undigested and unabsorbed starch will reach the colon where it may either contribute to faecal bulk and increase the dilution of potential carcinogens, or it may be fermented to short-chain fatty acids, which may subsequently decrease the colonic pH. The increased production of shortchain fatty acid, partic - ularly butyrate, may play a protective role in colon carcinogenesis, because butyrate has been shown in several in vitro studies to slow down the growth rate of human colorectal cancer cell lines [95,96]. Decreased pH has been suggested to be protective of colon carcinogenesis [97] by possibly causing alterations in the metabolic activity of colonic flora, altering bile acid metabolism and inhibiting ammonia production and absorption [98, 99].

IV. Conclusion

Phytate is a principal chelating agent in cereal-based foods and is capable of impairing divalent mineral bioavailability through binding. Phytate has been recognized as an antinutrient due to its adverse effects. It reduced the bioavailability of minerals and caused growth inhibition. Many studies reported that phytate in plant foods binds essential dietary minerals in the digestive tract, making them unavailable for absorption. It forms insoluble complexes with Cu2+, Zn2+, Fe3+ and Ca2+ and as a result reduces the bioavailability of these essential minerals. Many animal feedings of plant food trials reveal that lower bioavailability of zinc, calcium, magnesium, phosphorus and iron are due to the presence of phytate. This is the main reason why phytate has been considered as an antinutrient.

Recent studies on phytate have shown its beneficial effects such as decrease in blood lipids, decrease in blood glucose response and cancer risk. In addition, a high phytate diet is used in the inhibition of dental caries and platelet aggregation, for the treatment of hypercalciuria and kidney stones in humans, and as antidote activity against acute lead poisoning. The beneficial health effects of phytate are more significant for populations in developed countries because of the higher incidence of cancer especially colon cancer which is associated with higher fat and lower fibre rich food intakes. Such populations generally do not suffer from mineral deficiencies. On the one hand, the chelating ability of phytate is considered to be a detriment to one's health whilst, on the other hand, many researchers consider this ability to bind with minerals as its most powerful asset. Such a variant topic signifies that more intensive studies are needed to obtain better insight into the mechanism responsible for the ''friend or foe" challenge of phytate. Moreover, regardless of a series of researches on the positive and negative features of phytate, the information on the dosage for humans eliciting positive or negative effects is limited and the optimal dosage for clinical therapies is yet to be determined.

V. Acknowledgement

I acknowledge all the Authors I used as a refer - ences in preparing this review paper. The author have no conflict of interests.

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Global Journal of Medical research: K
Interdisciplinary
Volume 14 Issue 3 Version 1.0 Year 2014
Type: Double Blind Peer Reviewed International Research Journal
Publisher: Global Journals Inc. (USA)
Online ISSN: 2249-4618 & Print ISSN: 0975-5888

Comparison of Plasma Tumor Necrosis Factor Alpha (TNF-Alpha) Levels between Obese and Non-Obese With Graded Exercise

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Abstract- *Introduction:* Obesity is hazardous to health. On the other hand, performing exercises regularly has health benefits. The plasma cytokine levels get altered with exercise. Cytokines modulate the activity of immune system. Tumour Necrosis Factor alpha is a pro-inflammatory cytokine.

Methods: The effect of single bout of moderate exercise and a single bout of strenuous exercise and one month of regular moderate exercise on plasma TNF- **α** level was estimated. 24 healthy non-obese subjects (15 males and 9 females) with mean age, 20.81years and mean BMI; 21.49 ± 1.23 kg/m2 were recruited. 8 obese, but otherwise healthy individuals (5 males and 3 females) with mean age 20.92 years, mean BMI; 31.78 ± 3.38 kg/m2 were inducted into the study. Age range of subjects in both groups was 18-25 years. Standardized 10m Shuttle Walk Test regime was used for performing the exercise. Plasma TNF-**α** was measured by Sandwich ELISA technique. The reagent kit used was from Duoset ELISA Development System (R & D Systems Europe Ltd). The readings were taken at 450nm using Organon Teknika Reader 230S.

Keywords: *obese, non-obese, tumour necrosis factor alpha, exercise, inflammation.*

GJMR-K Classification: *NLMC Code: QZ 310*

Strictly as per the compliance and regulations of:

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Statistical methods: Repeated measures ANOVA was used to find the differences in TNF-α level with different grades of exercise. Post hoc tests using the Bonferroni correction was employed to compare the cytokine levels between the 2 groups.

Results: Amongst the non-obese, mean and SD values of TNF-α (in picograms per ml) for baseline (no exercise) was: 9.79 ± 1.15, for acute moderate exercise: 13.98 ± 2.66, for acute strenuous exercise: 48.28 ± 5.90 and after one month of regular moderate exercise: 5.89 ± 0.45. Amongst the obese, the TNF-α level were as follows: baseline; 14.57 ± 2.36, acute moderate exercise; 45.50 ± 15.77, acute strenuous exercise; 95.82 ± 4.16, and at end on one month of regular moderate exercise; 13.30 ± 2.76. TNF-α level showed significant difference between; a) baseline and moderate exercise, b) baseline and strenuous exercise, c) moderate and strenuous exercise, d) strenuous exercise and end of one month of regular moderate exercise, e) baseline and end of one month of regular moderate exercise, f) moderate exercise and end of one month of regular moderate exercise in both obese and non obese. The TNF-α level differed significantly between obese and non obese groups in each grade of exercise. TNF-α showed overall significance between different grades of exercise in both groups ($p < 0.05$).

Conclusions: The plasma TNF-α levels were higher in the obese group compared to the non-obese group in all grades of exercise. In both groups, plasma TNF-α increases with one bout of acute moderate exercise and increases further with one bout of acute strenuous exercise and decreases to below baseline value at end of one month of regular moderate exercise. This shows that regular moderate exercise has beneficial effects on health by way of decreasing TNF-α level.

Keywords: obese, non-obese, tumour necrosis factor alpha, exercise, inflammation.

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I. Introduction

Tumour Necrosis Factor alpha (TNF-α) is a pro-inflammatory cytokine [1,2]. Higher levels of inflammatory cytokines like TNF-α and IL-6 is positively correlated with the increased prevalence and complications of life style diseases [3, 4]. Obesity is also associated with increased incidence of metabolic syndrome and other life style disorders. Unaccustomed physical activity can have harmful effects on health [5]. It increases serum IL-6 levels and the hsCRP (highly sensitive C reactive protein) to correlate with increased incidence of cardiovascular diseases [6]. Persisting physical stress increases secretion of TNF-α and IL-6 which in turn leads to premature onset of lifestyle diso - rders [7]. Moderate exercise performed regularly decreases severity of inflammation in rheumatoid arthritis [8],[9]. The performance of immune system improves with daily practice of moderate exercise [10]. Regular moderate exercise improves overall health in all age groups [11],[12].

Scientists have observed the plasma cytokine changes with different modes of exercises like mara - thon, military training, downhill running on a treadmill, cycling, etc., on different groups of individuals in differ - rent parts of the world [13][14][15][16]. We under -took this study in order to understand the impact of moderate and strenuous exercise on the plasma levels of TNF-α in unaccustomed obese and non-obese individuals and the benefit of exercise on accustumisat –ion by the same individuals.

II. Materials and Methods

24 healthy non-obese subjects (15 males and 9 females) with mean age, 20.81years and mean BMI; 21.49 ± 1.23 kg/m2 were recruited. 8 obese, but otherwise healthy individuals (5 males and 3 females)

with mean age 20.92 years, mean BMI; 31.78 ± 3.38 kg/m2 were inducted into the study. Age range of subjects in both groups was 18-25 years. Subjects in both the groups were not performing any form of regular exercise. Prior consent was obtained before inducting them into the study. Clearance was obtained from the institutional ethical committee for the study. The approved number of subjects was 40.

The subjects in both groups were made to perform one bout of moderate exercise (acute moderate exercise), one bout of strenuous exercise (acute stre - nuous exercise) and one month of scheduled moderate exercise on a daily basis. The subjects were made to perform acute moderate exercise on the first day and acute strenuous exercise on the second day. They were made to perform scheduled regular moderate exercise from the third day onwards, for 30 days. The exercise was performed under supervision. During one month of scheduled moderate exercise, the subjects were made to perform single bout of moderate exercise daily for 30 days. The exercise was graded as moderate or strenu - ous based on the rise in heart rate. It was labelled as moderate when the heart rate increased by 50% from resting level and was labelled as strenuous when heart rate increased by 100%[18].

Shuttle Walk Test Protocol The exercise regime chosen was the standardized 10m Shuttle Walking test regime, described by Glenfield Hospital, Leicester, United King -dom in collaboration with the department of Physical Education and Sports Science, Lough - borough Univ -ersity of Technology, United King - dom[19][20][21] [22]. In this exercise protocol, the subjects walk on a 10 meter plain path at the two ends of which are placed marker cones. The subjects walk between the cones corresponding to the beeps given out by a record player. Subjects have to increase their speed of walking gradually in tandem with the shorte - ning of intervals between the consecutive beeps as time progresses. The level of the shuttle walk regime at which the heart rate increased by 50% of the baseline was chosen as moderate exercise. The level at whic h the heart rate increased by 100%, i.e. doubled was consi - dered as strenuous exercise.

A venous blood sample from cubital vein (using vacutainers) just before acute moderate exercise (bas - eline) was collected. Another sample was collected immediately after acute moderate exercise on the same day. After performance of acute strenuous exercise on the next day, third sample was obtained. A sample was obtained after one month of scheduled regular mod - erate exercise on the last day after exercise. Baseline sample just before acute strenuous exercise, and just before performance of exercise on the last day of one month regular moderate exercise was not obtained. The samples collected from each individual were aliquoted and stored at - 400C till analysis.

Plasma sample was used to estimate the level of TNF-α, by using ELISA (Enzyme linked Immun - oSorbent Assay) method. ELISA was performed using DuoSet ELISA development system as per the manu - facturer's instructions (R&D systems, USA).

Estimation of TNF- α:

Polystyrene microtiter plates (NUNC, U16 Maxisorp type, Denmark) were coated with monoclonal capture antibody (antihuman TNF- α) obtained from mouse (R&D systems, USA) and incubated at 4°C overnight. The following day, the plates were blocked and then incubated for 2 hours with plasma. This was followed by addition of corresponding biotinylated detection antibody obtained from goat (R&D systems, USA) and incubated for 2 hours. Strepatavidin, horser - adish peroxidise conjugate and then, 3,3',5,5'- tetramet - hylbenzidine substrate (Bangalore Genie, India) followed this incubation. The reaction was stopped using 2 N sulphuric acid and optical density (O.D) reading was taken at 450nm (Organon Teknika Microwell system, Reader 230s, Germany). All the experiments were conducted in duplicates. A standard curve was obtained based on the standards provided by the manufacturer. The results were expressed as concentration of cytokines (in pg/ml) read from the standard curve (concentration in range: minimum of 5 pg/ml, to maximum of100 pg/ml).

a) Statistical Analysis

Data was entered in M S Excel and was analyzed using SPSS Version 20.0 (SPSS Inc. Chicago, USA). All the continuous variables were summarized in terms of mean and standard deviation and categorical variables as proportions. In order to test for statistical significance for differences in the mean values of TNF-α at different time points (i.e.; during various grades of exercise), in each group (obese and non-obese), repeated measures of ANOVA was employed. Further, pair wise differences were tested using Bonferroni's test. Pearson's corre -lation coefficient was used to find the correlation between BMI and TNF-α in both groups.

III. Results

8 obese and 24 non-obese individuals took part in the study. Plasma TNF-α level was studied with different grades of exercises. Among the non obese, 15 (62.5%) were males and 9 (37.5%) were females. Amo - ng the obese, 5 (62.5%) were males and 3(37.5%) were females. The mean BMI was 21.49 ± 1.23 kg/m2 among the non-obese and 31.78 ± 3.38 kg/m2 among the obese.

A repeated measures ANOVA determined that mean TNF-α levels differed statistically significantly between the various exercise levels in obese group and non-obese group ($P < 0.01$). Post hoc tests using the Bonferroni correction revealed that exercise elicited

decrease in TNF-α concentration in obese [19 ± 0.54 (Mean± SEM)] and non-obese group [(42.30 ± 0.94 (Mean± SEM)] which was statistically significant ($p < 0.01$). Therefore, we can conclude that a long-term exercise elicits a statistically significant reduction in TNF-α level.

There was a significant increase in the levels of this cytokine with both acute moderate exercise ($p=0.003$ and $p=0.002$ in obese and non-obese respe - ctively) and acute strenuous exercise ($p=0.005$ and $p=0.003$ in obese and non-obese respectively) comp - ared to baseline value. There was a significant rise in its levels after acute strenuous exercise when compared to moderate exercise ($p=0.043$ and $p=0.002$ in obese and non-obese respectively). The fall of TNF- α after one month of regular moderate exercise was also significant compared to baseline value ($p=0.001$ and $p=0.001$ respectively). That is, the TNF-α level decreased to below baseline level after the bout of moderate exercise on the last day of one month of regular moderate exercise regime in both groups (Table: 1, Figures: 1, 2).

Table 1 : Comparison of mean TNF alpha levels during various grades of exercise in obese and non obese group

	Group	Mean ± SD
TNF-α at baseline	Non-obese	9.79 ± 1.15
	Obese	14.57 ± 2.36*
TNF-α after a bout of moderate exercise	Non-obese	13.98 ± 2.66
	Obese	45.50 ± 15.77*
TNF-α after a bout of strenuous exercise	Non-obese	48.28 ± 5.90
	Obese	95.82 ± 4.16*
TNF-α after 1 month regular moderate exercise	Non-obese	5.89 ± 0.45
	Obese	13.30 ± 2.76*

**TNF-α alpha in pg/dl*

n=24 for obese and n=8 for non obese

**p < 0.05: TNF- is statistically significant between different grades of exercise and between obese and non-obese groups.*

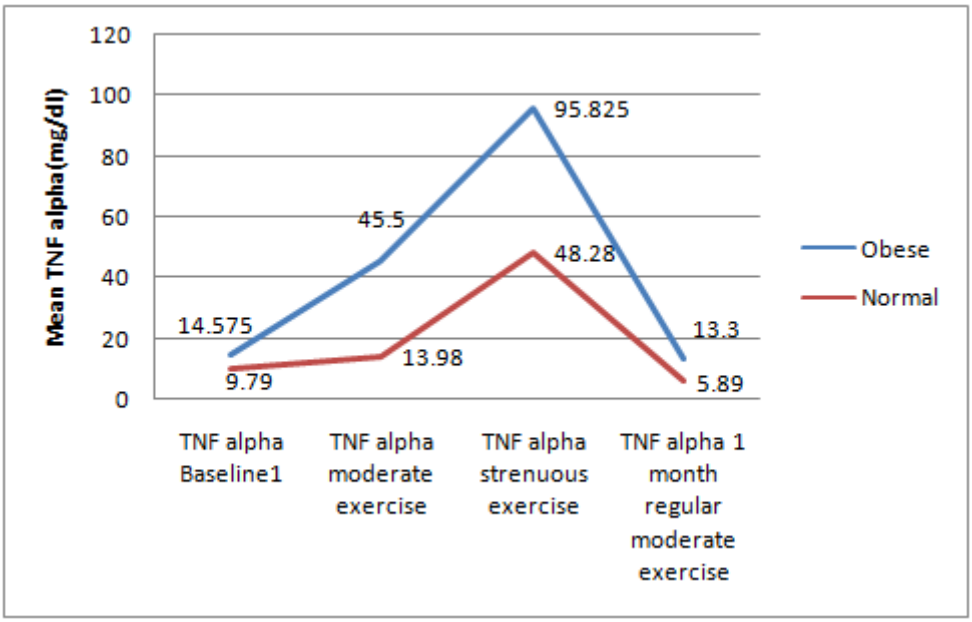

Figure 1: Changes in the TNF-α level (pg/ml) in obese and non-obese (normal) groups with different grades of exercise

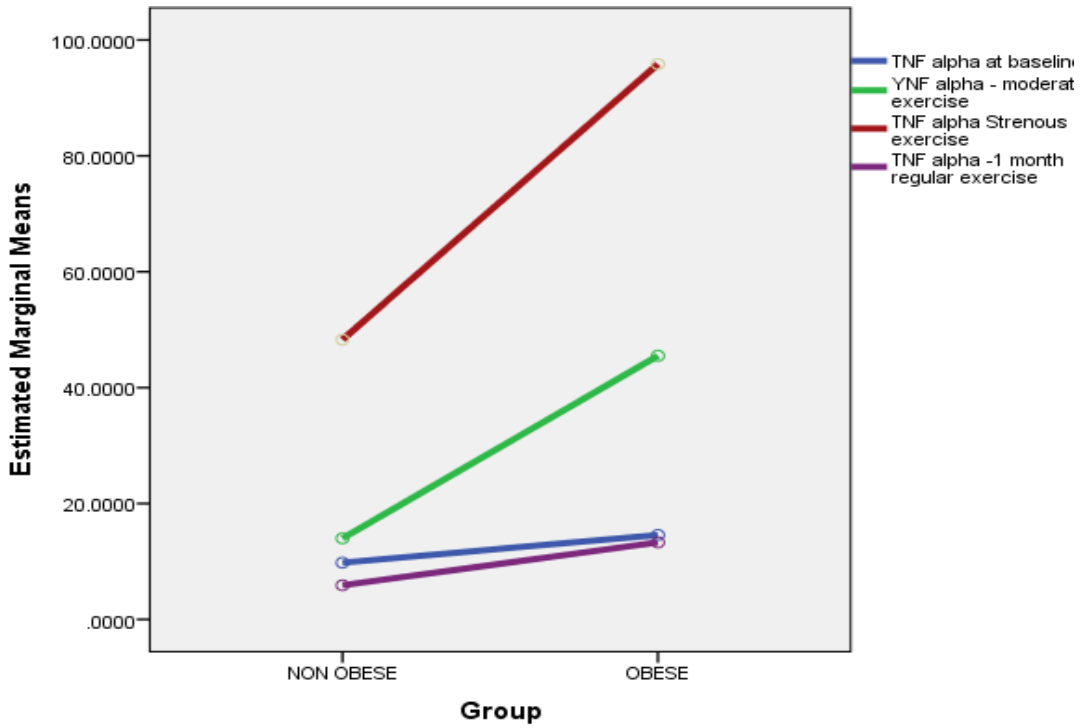

Figure 2 : Comparison of TNF-α level (pg/ml) between obese and non-obese at different grades of exercise

There was a positive correlation in both obese and non-obese groups at baseline (no exercise) but it was not statistically significant in both groups. It was found that BMI had a significant positive correlation with TNF-α in both obese and non obese groups but the correlation was high ($r=0.975$, $p<0.001$)) in obese as compared to non obese group ($r=0.76$, $p<0.05$)) after a bout of moderate exercise. There was a positive correlation of BMI and TNF-α alpha during strenuous exercise in obese($r=0.59$) which was not statistically significant. There was correlation between BMI and TNF-α in non-obese group for strenuous exercise but it was not statistically significant ($r=0.16$). There was a negative correlation between TNF-α and BMI after one month of regular moderate exercise, but it was not statistically significant in both obese ($r=-0.25$) and non-obese ($r=-0.17$) groups.

IV. Discussion

Obesity is a health hazard. This study was undertaken to see if there is any difference in the behavior of plasma levels of the pro-inflammatory cytokine TNF-α, when the obese subjects and non-obese subjects were made to undergo identical physical stress. Sudden and excessive physical activity is hazardous to health [23]. Physical injury and unacc - ustomed physical stress/exercise has similar effects on immune system. [24]. There is production of pro-inflammatory cytokines when the human body is made to undergo acute physical exercise. [25],[26]. The percentage of T cells decrease in circulation on perfo - rmance of long term severe exercise. [27]. Regular practice of moderate exercise is inversely correlated with levels of pro-inflammatory cytokines in coronary heart disease patients retarding the process of athero - sclerosis [28]. Therefore, higher levels of pro-inflam - matory cytokines like tumor necrosis factor alpha are harmful to health [29].

TNF-α has pro-inflammatory properties. In this study, in both the obese and the non-obese groups, TNF-α levels increased after a bout of moderate exercise and there was a further significant increase following a bout of strenuous exercise and decreased significantly compared to baseline levels when comp - ared with one month of scheduled regular moderate exercise done on a daily basis; that is, in both the groups, TNF-α levels decreased to below baseline level after the single bout of moderate exercise on the last day of one month of scheduled moderate exercise when compared to single bout of moderate exercise without accustumisation to regular moderate exercise, in the same individuals. It can be noted that the TNF-α levels are higher in obese subjects at baseline (no exercise) level as well as at all other grades of exercise. In those subjects who perform moderate exercise on a daily basis, sudden increase in pro-inflammatory cytokine may not occur if such individuals were to perform severe bouts of unaccustomed physical activity intermittently. This may help them to tolerate sudden and unaccu - stomed physical stresses in life better than those who do not exercise regularly..

The immune status improves markedly with regular moderate exercise [6][7][8][10][11]. Since this study shows a fall in TNF-α level with regular moderate

exercise in both groups, a fall in TNF-α level should also be beneficial for maintaining health and immunity. TNF-α is pro-inflammatory cytokine, so its altered production leads to unnecessary inflammation and tissue damage [30]. Thus regular moderate exercise seems to mod - ulate its release and alters its levels to the optimum levels necessary for human body to maintain good health.

Mental stress is also known to increase the level TNF-α [31]. The adaptive cytokine response may also help individuals adhering to regular moderate exercise to cope with bouts of psychological stresses enco - untered in daily life [32]. The levels of TNF-α may not rise drastically either [33].

Elevated levels of TNF-α interleukin-6 (IL-6) are observed in atherosclerosis, coronary artery disease and diabetes mellitus, etc [30]. Stressful bursts of physical activity in daily life, in such patients increases their levels much further and leads to exacerbation of the disease. It can be postulated that the drastic rise in TNF-α and IL-6 with bursts of physical activity or with 'acute on chronic infections' tends to become mild if such patients perform moderate exercises regularly.

Certain autoimmune disorders like systemic lupus erythematosus and rheumatoid arthritis are associated with increased plasma levels of pro inflammatory cytokines like TNF- α and IL-6, which increased inflammation [34]. Increased levels of TNF-α leads to cachexia, increased levels of C-reactive protein and other acute phase proteins, activates macrop - hages, increases tumour cytotoxicity, activates neutr - ophils and increases phagocytosis and induces secre - tion of other pro-inflammatory cytokines like IL-6 [36]. This study shows a positive correlation between TNF-α and BMI baseline (no exercise) though not statistically significant in both obese and non-obese groups. It was found that BMI had a significant positive correlation with TNF-α in both obese and non obese groups but the correlation was high ($r=0.975$, $p<0.001$)) in obese as compared to non obese group ($r=0.76$, $p<0.05$)) after a bout of moderate exercise. There was a positive correlation of BMI and TNF-α after strenuous exercise in obese ($r=0.79$, $p<0.05$) which was statistically significant. This demonstrates that the obese are more prone to secrete higher levels of pro-inflammatory cytokines like TNF-α on stressful physical activity to which they are not accustomed. There was correlation between BMI and TNF-α in non-obese group for strenuous exercise but it was not statistically significant ($r=0.16$). There was a negative correlation between TNF-α and BMI after one month of regular moderate exercise, but it was not statistically significant in both obese ($r=-0.25$) and non-obese ($r=-0.17$) groups..This may indicate that obesity predisposes to increased levels of pro-inflammatory cytokines, especially when the obese are exposed to unaccustomed physical stress. Interestingly, we found a negative correlation between BMI and TNF-α, though not significant at end of one month of regular moderate exercise in both groups. This may be because of the increase in healthy lean body mass/muscle mass at end of one month of exercise and decrease in adiposity [37]. Till date very few studies have been undertaken simultaneously in the obese and non-obese groups of human subjects to study the effects of physical stress/exercise on plasma level of TNF-α. One of the reasons for this may be that it is difficult to convince obese subjects to perform physical exercises, especially under supervision, which are both physically and psychologically stressful for them [38]. Obesity associated inflammation is a known entity, but the mechanism controlling this pathway is still being investigated and is not clearly known [39]. Regular moderate exercise may not only benefit obese individuals but also those patients suffering from disorders related to metabolic syndrome like diabetes, inflammatory diseases and auto-immune disorders by bringing down the levels of pro-inflammatory cytokines like TNF-α. Since the behavior of plasma TNF-α level differs in obese and non-obese subjects with different grades of physical exercise, we propose that this study has potential for clinical application.

V. Acknowledgements

We would like to acknowledge the support and valuable inputs of Dr. Chandrashekara. S, Director, Chanre Rheumatology and Immunology center for Research, Bangalore and Dr. Rajeev Sharma, former head, Department of Physiology, M. S. Ramaiah Medical College, Bangalore, India.

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Global Journal of Medical research: K
Interdisciplinary
Volume 14 Issue 3 Version 1.0 Year 2014
Type: Double Blind Peer Reviewed International Research Journal
Publisher: Global Journals Inc. (USA)
Online ISSN: 2249-4618 & Print ISSN: 0975-5888

Post Control Survey on Prevalence of Bovine Trypanosomosis and Vector Distribution in Ameya District, South West Shewa, Ethiopia

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Abstract- A cross sectional study was conducted from February to June, 2013 to determine prevalence of bovine trypanosomosis and population of tsetse and other biting flies, and to assess effects of integrated control strategy implem - ented in the last five years in Ameya district, South West Shewa, Ethiopia. Both primary and secondary data were used. Structured questionnaire survey was conducted by face to face discussion. Buffy coat technique was used for screening followed by thin smear technique for trypanosome species identification. Baited monopyramidail traps were deployed at a distance of 300m apart for 72 hours to catch flies. The district was identified as one of the areas affected by bovine trypanosomosis and infested by tsetse and other biting flies. Integrated control strategy has been implemented to reduce occurrence of the disease and its vectors. From 436 examined animals, 6 (1.4%) were positive. Two species of trypanosome, Trypanosoma brucei (3/6, 50%) and Trypanosoma congolense (2/6, 33.33%) were identified in a single infection while 16.67% (1/6) mixed infection of both species was obtained. There was no statistical significance difference ($p>0.05$) in prevalence of the disease among groups of peasant associations and age, and between sex groups. The mean packed cell volume of parasitemic and aparasitemic cattle was similar.

Keywords: *Ameya district, biting flies, bovine trypano - somosis, integrated control strategy, tsetse flies.*

GJMR-K Classification: *NLMC Code: WT 30*

Strictly as per the compliance and regulations of:

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Abstract- A cross sectional study was conducted from February to June, 2013 to determine prevalence of bovine trypanosomosis and population of tsetse and other biting flies, and to assess effects of integrated control strategy implemented in the last five years in Ameya district, South West Shewa, Ethiopia. Both primary and secondary data were used. Structured questionnaire survey was conducted by face to face discussion. Buffy coat technique was used for screening followed by thin smear technique for trypanosome species identification. Baited monopyramidail traps were deployed at a distance of 300m apart for 72 hours to catch flies. The district was identified as one of the areas affected by bovine trypanosomosis and infested by tsetse and other biting flies. Integrated control strategy has been implemented to reduce occurrence of the disease and its vectors. From 436 examined animals, 6 (1.4%) were positive. Two species of trypanosome, *Trypanosoma brucei* (3/6, 50%) and *Trypanosoma congolense* (2/6, 33.33%) were identified in a single infection while 16.67% (1/6) mixed infection of both species was obtained. There was no statistical significance difference ($p>0.05$) in prevalence of the disease among groups of peasant associations and age, and between sex groups. The mean packed cell volume of parasitemic and aparasitemic cattle was similar. A total of 6236 biting flies, including 6133 (98.35%) *Stomoxys*, 18 (0.29%) *Tabanus*, 14 (0.22%) *Chrysops* and 71 (1.14%) *Haematopota* were captured. However, tsetse fly was not captured. Statistical significance difference was observed in mean catch of *Stomoxys* and *Tabanus* ($p<0.05$) in different peasant associations. In conclusion, application of integrated control strategy undertaken in the district resulted in reduction of disease occurrence and tsetse flies. Thus, continuation of the ongoing integrated control strategy and dissemination of the strategy to neighboring districts to limit re-invasion with tsetse flies were recommended.

Keywords: Ameya district, biting flies, bovine trypanosomosis, integrated control strategy, tsetse flies.

I. Introduction

Livestock are of enormous importance socially and economically for nutritional and agricultural purposes in Africa (Uilenberg, 1990). In developing countries, diseases of livestock reduce agricultural output by up to 30% (FAO, 1990). Among animal diseases, presence of trypanosomosis, which is caused by trypanosome and transmitted cyclically by tsetse flies or mechanically by biting flies (Maudlin *et al.*, 2004; Radostits *et al.,* 2007), is a major constraint on agricultural production and has a devastating effect on livestock and man. Tsetse flies are largely responsible for an uneven distribution of cattle in Africa, leading to overgrazing and severe environmental degradation in some areas and preventing the introduction of productive farming and livestock systems in other areas. Tsetse and trypanosomosis are problems that are closely linked with rural poverty, thus, tsetse fly is frequently referred to as the "poverty insect" (IAEA, 2003). It is also responsible for an annual loss of millions of dollars in livestock production as a result of the cost related to treatment, prevention and vector control efforts (Samuel *et al.,* 2001) and death of animals (Bett *et al.,* 2004).

Control of the disease can be based on control of the causal agent and its vector, and use of innate resistance of the host to the effects of the infection (Uilenberg, 1990). There are several methods that may be used to try to reduce trypanosomosis and its effects; a combination of these methods will be used in any campaign carried out on a continental scale (Pollock, 1982). In Ethiopia, several attempts have been made to control the disease with chemotherapy and chemoprophylaxis being the most widely applied methods. Vector targeted control practices have been implemented mainly through specifically designed joint projects of the Ministry of Agriculture and other non-governmental organizations (Tafese *et al.,* 2012).

As part of the Gibe river system, tsetse-transmitted animal trypanosomosis has been incriminated as the primary disease condition to highly curtail the production potential of its livestock sub-sector, particularly in the fertile lowland and midlands of Ameya district, South West Shewa zone, Ethiopia. The district was one of the highly affected areas by animal trypanosomosis and infested by different tsetse and biting flies (NTTICC, 2009; Denu *et al.*, 2012). Since intense studies in 2007/2008 in the district, integrated control strategy has been undertaken by support of governmental and non-governmental organizations. Thus, knowing the current status of the disease and its vectors is crucial to apply efforts towards combating the disease and reducing economic losses in the area. Therefore, the current study was conducted to determine the prevalence of the disease and its vectors, identification

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of vector flies and trypanosome species distributed in the area and to check the effectiveness of the undertaken control methods.

II. Materials and Methods

a) Study area

The study was conducted in Ameya district, South West Shewa zone, Ethiopia from February to March, 2013 (for prevalence of trypamosomosis survey) and from May to June, 2013 (for tsetse and biting flies survey). Gindo is the administrative town of the district and located at about 145 Km distant southwards of Addis Ababa, capital of Oromia region and Ethiopia. The district consists of midland and lowland situated in Gibe watershed, one of main rivers of Gibe-Omo River sys - tem. The district has 39 kebeles (peasant associations (PAs)), of which 19 (47%), 10 (36%) and 7 (17%) PAs are lowland, midland and highland, respectively. Five PAs having different sites, Gambela Ashute Talgo (Ashute site), Gombore Aliyi (Gombere, Eteya and Gombore Baticha sites), Mari Kereyu Sekela (Mari-Magari and Mari sites), Moko Ujuba Kota (Kota site) and Cha'a Kase (Atnafo site) were randomly selected for the study.

The topography of the areas is mainly plain and thoroughly cultivated. Mixed livestock and crop farming is the dominant form of production where rain-fed agriculture is the common production system. The vegetations are confined on the river sides, and the types of vegetations are dominated by thick and scattered thickets with short grasses on the upper reaches of the rivers. As inhabitants of the area are cutting down the trees for fire wood and charcoal, the vegetation is getting thinner and thinner. The wild life could not be found in a group due to lack of enough shelter as needed except some wild games.

The district has a total of 15,715 local and 37 cross breed cattle. In addition, there are 30,881, 34,815, 7,803, 3,150, 13,046 and 85,823 sheep, goats, horses, mules, donkeys and poultry, respectively (Socio-economic data, 2012).

b) Study protocol

Both primary and secondary data were used. The primary data were collected from field works. Secondary data were obtained from Livestock Devel - opment and Health Agency of Ameya district after provision of permission request letter, and published sources on works conducted in the district before five years. The obtained secondary data include prevalence of the disease, distribution and densities of tsetse and biting flies before five years, and control strategies applied in the last five years.

c) Questionnaire survey

A structured questionnaire survey was conducted to gather information on aspects of livestock management, socio-economics of the society, trypano - somosis and its vectors, and control strategies being used to control the disease and its vectors. Fifty-one respondents were randomly selected from the house - holds, who are residents of the district, and informed about purpose of the study. The questionnaire was administered by face to face discussion.

d) Study design and animals

A cross sectional study was conducted on 436 randomly selected local zebu breed cattle of both sexes. Age of animals was determined using owners' infor - mation. Accordingly, animals were categorized as young (<2 years), young adult (≥2<5 years), adult (≥5≤9 years) and old (>9 years). Entomological survey was conducted in selected areas that seemed to be habitat for tsetse flies using monopyramidal traps.

e) Parasitological survey

A small quantity of paired blood samples were collected into heparinized haematocrit capillary tube (filled up to about ¾) from marginal ear vein by pricking with the tip of a sterile lancet after properly securing the animal and aseptically preparing the area. Buffy coat technique was used. The samples were centrifuged at 12000 rpm for 5 minutes. The capillaries were measured by haematocrit reader and a packed cell volume (PCV) value of each cattle was recorded.

The capillary tube was cut 1 mm below the buffy coat using diamond pencil. The content of the capillary tube was expressed onto a clean microscope slide and covered with a 22x22 mm cover slip. Then the slide was examined for trypanosomes based on the type of movement in the microscopic field with 40X objective lens magnification. Confirmation of trypanosome spe - cies of positive samples by morphological charact - eristics was performed by thin smear technique and examination by light microscopy of 100X objective lens magnification (Murray et al., 1977; Uilenberg, 1990).

f) Entomological survey

During entomological survey, 24 monopyram-idal traps were deployed in selected sites (Gombore, Mari Magari, Mari and Kota sites) at a distance of about 300 m apart for 72 hours. Every trap was odour-baited with acetone (150 mg/hr release rate), 3-octen-1-ol (0.5 mg/hr release rate) and cow urine (1000 mg/hr release rate) (Terfa *et al.*, 2014). The underneath of each trap pole was smeared with grease in order to prevent the ants climbing up the pole towards the collecting cage that could damage the flies.

After a specified period of deployment, the flies caught in the collecting cage were sorted, identified, counted and the apparent fly density per trap per day was recorded. A hand lens was used for the identif-ication flies based on the characteristic morphological structures at genus level.

g) Data analysis

All the collected data were coded and entered into the Microsoft Excel spreadsheet and analyzed using

Statistical Package for the Social Sciences (SPSS) 16.0 version. Frequency distribution and percentage were used to summarize data obtained from questionnaire survey and prevalence of the disease. Vector survey data were analyzed using student t-test and ANOVA to compare the mean catches in different study areas. In all cases, 95% confidence interval was employed.

III. Results

a) Secondary data

Previous study was conducted by NTTICC (National Tsetse and Trypanosomosis Investigation and Control Center) in April, 2008 and reported in 2009 in two peasant associations (Mari Magari and Bareda Chilalu) before implementation of integrated control strategy. A total of 16 and 12 *Trypanosoma congolense* (*T. congolense*) and *Trypanosoma vivax* (*T. vivax*) were isolated from 192 examined cattle in the district with 11.45% and 17.7% prevalence rate in Mari Magari and Bareda Chilalu, respectively. Meanwhile, 40 monopyramidial traps were deployed and 28 *Glossina pallidipes*, 658 *Stomoxys* and 5 *Tabanus* flies were captured.

According to information from Livestock Development and Animal Health Agency of Ameya district, a total of 380 liters undiluted 18.75% deltamethrin were provided and poured on 25,350 cattle and 225 targets impregnated with 0.5% deltamethrin were deployed in the last adjacent five years (2007-2013) for 10 kebeles (PAs) in the district (Table 1). In addition, trypanocidal drugs and deltamthrin provided to the cattle owner by public and private veterinary clinics and pharmacies were also contributed for control activities and constitute the utmost proportion.

Table 1 : Trypanosomosis control mechanisms used in the last five years in Ameya district

Year	Poured on deltamethrin (liter)	Number of cattle sprayed	Target deployed
2007/08	100	5000	-
2008/09	170	7000	100
2009/10	50	4000	100
2011/12	70	4100	25
2012/13 (up to May, 2013)	90	5250	-
Total	380	25,350	225

b) Primary data

i. *Questionnaire survey*

The questionnaire survey result indicated that about 100% of the respondents practice mixed (livestock-crop production) farming system. Additionally, 9.8% (5/51) respondents participate in trade and feedlot activities. Cattle are reared as a source of milk, meat, manure, drought power, income generation, paying dowry, wealth banking, bleeding (sacrifice) and fattening.

Free grazing was the common practice (40/51 respondents (78.4%)) while free gazing combined with stall feeding during milking for lactating cows and drought oxen was also rarely used (11/51 (21.6%)). Crop residues like straws and residues of local beer called *"atalaa"* were identified as source of feed for stall feeding. Majority of the respondents (32/51 or 62.5%) were using bush and grassland areas to graze their animals, which are found either nearby the river bodies or not (Table 2).

The disease was first (49/51 respondents or 96%), second and fourth (1/51 respondent or 2% each) ranked among known animal diseases and resulted in death of 21 cattle owned by some of the 51 respondents in the last 12 months. From 51 respondents, 36 (70.6%) and 13 (25.5%) claimed decrease of milk production by 100% and 50%, respectively in lactating cows while all of them agreed on decreasing by half to complete cessation of drought power of oxen due to the disease. Effects of the disease like abortion, delay of first calving, long calving interval and birth of underweight calves were common in the area.

Majority of the respondents (37/51 (72.5%)) claimed as the disease was introduced in the district in 1970s while the rest (14/51 (27.5%)) said 20 years ago. About 90.2% (46/51) of the respondents responded as occurrence of the disease is decreased over last five years while the rest 9.8% (5/51) respondents claimed increased occurrence of the disease. However, respondents in both groups know and agreed on different control strategies performed in the district except 2 (3.9%) respondents (Table 2).

Table 2 : Summary of questionnaire survey on trypanosomosis and its vector in Ameya district

Variables	Total respondents	Frequency	Proportion (%)
Grazing area			
Grassland	51	11	21.6
Bush area	51	2	3.9
Bush and grassland	51	32	62.5
Bush area and bottom of valley	51	4	7.8
Grassland and top of valley	51	1	2
Bottom of valley	51	1	2
Season of occurrence of trypanosomosis utmost			
Wet season	51	9	17.6
Dry season	51	37	72.5
The same throughout a year	51	5	9.8
Know tsetse fly			
Yes	51	19	37.3
No	51	13	25.5
Know other vectors of trypanosomes	51	17	33.3
Know tsetse and other biting flies	51	2	3.9
Season of tsetse and other flies that transmit trypanosomosis abundance			
Wet season	51	38	74.5
Dry season	51	6	11.8
The same throughout a year	51	1	2.0
Don't know	51	6	11.8
Level of trypanosomosis in the last five years			
Increased	51	5	9.8
Decreased	51	46	90.2
Applied control strategies in the area			
Trypanocidal drugs	51	7	13.7
Trypanocidal drugs and trap/target	51	7	13.7
Trypanocidal drugs and pour on	51	12	23.5
Trypanocidal drugs, trap/target and pour on	51	21	41.5
Trypanocidal drugs, pour on and others (deforestation to cultivate the land, supplementary feeding)	51	2	3.9
Don't know any applied method	51	2	3.9

ii. *Prevalence of trypanosomosis*

In the current study, an overall 1.4% (6/436 cattle) prevalence of the disease was recorded. There was no statistical significance difference between sex ($p>0.05$), among PAs and age ($p>0.05$) groups (Table 3). Two species of trypanosomes, *T. congolense (*2/6 or 33.33%) and *Trypanosoma brucei (T. brucei)* (3/6 or 50%) were identified as a single infection and mixed infection of *T. congolense* and *T. brucei* (1/6 or 16.67%).

Table 3 : Prevalence of trypanosomosis in groups of different risk factors

Variable	Category	No. of examined	No. of positive (%)	Fishers' exact	p-vlue
PAs	Gambela Ashute Talgo	47	1(2.13)		
	Gombore Aliyi	129	2(1.55)		
	Mari Kereyu Sekela	115	3(2.61)	2.801	0.532
	Moko Ujuba Kota	73	0		
	Cha'a Kase	66	0		
Sex	Male	209	3 (1.44)	0.01	1.000
	Female	227	3 (1.32)		
Age (years)	<2	27	0		
	≥2<5	150	2 (1.33)	0.312	1.000
	≥5≤9	231	4 (1.73)		
	>9	28	0		

There was no observable difference in the mean PCV of the aparasitemic and parasitemic cattle. However, the mean PCV of parasitemic cattle are slightly higher. The mean PCV of aparastemic cattle was 25.20

± 4.210 SD with a range of 11-38 while the mean PCV of parasitemic cattle was 25.50 ± 4.889 SD with a range of 19-31.

iii. *Entomological survey*

A total of 6,236 biting flies, of which 6,133 (98.35%) *Stomoxys*, 18 (0.29%) *Tabanus*, 14 (0.22%) *Chrysops* and 71 (1.14%) *Haematopota* were captured. However, no tsetse fly was captured. The apparent densities (fly/trap/day) of 85.18, 0.25, 0.19 and 0.97 were obtained for *Stomoxys, Tabanus, Chrysops* and *Haematopota*, respectively. The overall mean apparent density of the captured biting flies was 86.61 ± 65.72 SD.

Highest mean catch was recorded for *Stomoxys* (255.54 ± 196.648 SD) followed by *Haematopota* (2.96 ± 3.014) while the lowest mean catch was obtained for *Chrysops* (0.58 ± 1.018 SD) followed by *Tabanus* (0.75 ± 1.452 SD). Statistically, significance difference was obtained in the mean catch of *Stomoxys* ($F=4.777$, $p<0.05$) and *Tabanus* ($F=0.45$, $p<0.05$) among diff - erent PAs (Table 4). Further analysis revealed prese - nce of statistical significance difference between Gom - bore and Mari Magari, and Gombore and Kota for *Stomoxys,* and between Gombore and Mari and Gom - bore and Kota for *Tabanus*.

Table 4 : Significance of mean catch of biting flies using ANOVA test

Variable	F-test	p-value
Stomoxys	4.777	0.011
Tabanus	3.204	0.045
Chrysops	0.976	0.424
Haematopota	0.405	0.751

IV. Discussion

a) Questionnaire survey

Dependence of the community on mixed farming system is consistent with previous work of Denu *et al*. (2012) in the same area. Livestock are reared for different purposes, which are integral part of agricultural activity and used as a source of milk, meat, manure, drought power, income generation, wealth accumulation (as a bank), for dowry payment and worshiping by scarifying or bleeding the animal. However, livestock production has been hindered by many devastating animal diseases, of which trypanososmosis is the first ranked disease by majority (49/51, 96%) of the respo - ndents. The current result agrees with the pervious result in the area (Denu *et al.,* 2012) and work of Terfa (2008) in Gawo-Dalle district, Kellem Wollega zone, Ethiopia, in which all the respondents ranked trypanosomosis as first. About 72.5% (37/51), who are elders, agreed as the disease was first introduced and recognized in the area in 1970s while the rest, younger and immigrants, estimated 20 years ago. Similarly, 100% indigenous habitants of in Gawo-Dalle district, Ethiopia claimed as the disease was introduced in 1960s (Terfa, 2008).

Twenty one cattle owned by some of the respondents were died due to the disease in the last 12 months (from date of the interview). Abortion, delay of first calving, long calving interval and birth of under - weight calves, reduction in milk production and drought power of oxen were also the important in the area, which led to struggle with subsistence livelihood of the community and consistent with Maudlin *et al*. (2004). According to Vreysen (2006), the disease is among well known livestock production constraints in Africa, mainly in rural poor community and considered as a root cause of poverty. The overall economic loss caused by the disease was estimated to be US $1408-1540 million per annum (NTTICC, 2006).

About 72.5% (37/51) of the respondents said that the disease occurs mostly in dry season. This disagrees with the finding of Denu *et al*. (2012) who reported higher prevalence of the disease in wet season than dry season in the area. The difference may be due to observable effects of the disease during dry season in combination with feed shortage and concurrent diseases.

Majority of the respondents (37.3% or 19/51) know tsetse flies, 33.3% (17/51) knows other biting flies that can transmit the disease while 3.9% (2/51) knows both biting and tsetse flies. About 74.5% (38/51) said both tsetse and biting flies are abundant during wet season. According to the respondents, these flies are responsible to transmit the disease during watering and grazing in forest, grassland, bush area, bottom and top of valleys of different vegetation types. The current findings are consistent with work of Denu *et al*. (2012).

b) Prevalence

In the current study, prevalence of the disease was 1.4%. However, in 2007/2008 higher prevalence rate of 33.5% and 17.83% in the late rainy and dry seasons, respectively was recorded in three districts of South West Shewa (Denu *et al.,* 2012) from which two were included in the current study. In addition, higher prevalence of 11.45% and 17.7% was obtained in Mari Magari and Bareda Chilalu sites of Ameya district in 2008 (NTTICC, 2009). This indicated the decreased occurrence of the disease over the last five years and is consistent with response of the majority of the respo - ndents (46/51, 90.2%). Similarly, study conducted in two

areas (controlled and non-controlled area) indicated lower prevalence of the disease in the controlled (9.1%) than in non-controlled area (15.1%) in Hawa Gelan district of Kellem Wollega zone, Ethiopia (Fantahun *et al.,* 2012).

The difference between the current and previous studies in the same area is due to application of integrated control strategy and professional interv - ention recommended from the previous works. These control methods include treatment with trypanocidal drugs, deploying of impregnated targets, applying pour on of delthamethrine and increased clearance of the tsetse fly habitat for cultivation by the community. In addition, frequent treatment of the infected cattle and season of the study, dry season, might be reasons for lower result of the current study.

Two species, *T. congolense* and *T. brucei*, were isolated in the current study. *Trypanasoma brucei* was the dominant species, 50% (3/6) as single infection and 16.67% (1/6) as a mixed infection with *T. congolense*. However, *T. congolense* and *T. vivax* were the only reported species in the previous two studies of the same area before five years with *T. congolense* as the domi - nant species (NTTICC, 2009; Denu *et al*., 2012). In contrast to the current result, *T. brucei* was the least prevalent species isolated in some parts of Ethiopia while *T. congolense* and *T. vivax* were the dominant (Abebe and Jobre, 1996; Abebe, 2005; Mulaw *et al*., 2011; Tafese *et al*., 2012). The difference might be attributed to sensitivity of tests and delayed examination of buffy coat smear (Uilenberg, 1990).

Young adult (≥2<5 years) and adult (≥5≤9 years) were more susceptible to the disease than young (<2 years) and old (>9 years) cattle. However, there was no statistical significance difference among different age groups. The higher prevalence was observed in adult (1.73%) followed by young adult (1.33%). The current result is slightly in line of agreement with work of Denu *et al*. (2012) in which 16%, 22% and 24% infection rate was reported in calves (<1year old), adult (1-4 years old) and older animals (>4 years old), respectively in dry season with absence of statistical significance difference among age groups. This could be associated to the fact that adult cattle travel long distance for grazing and draught as well as harvesting crops in areas of high tsetse challenge (Denu *et al.,* 2012). According to Rowlands *et al*. (1995), sucking calves do not go out with their dams but graze at homesteads until they are weaned off, which is also true for this study area. Furthermore, protection of young animals by natural maternal antibodies to some extent (Fimmen *et al*., 1992) could be considered as one factor for low prevalence in young cattle.

There was no statistical significance difference between sex groups even though the prevalence of the disease was slightly higher in males than females. This is consistent with work of Tafese *et al*. (2012). However, Zecharias and Zeryehun (2012) reported slight higher prevalence in female cattle with absence of statistical significance difference in Arba Minch, Ethiopia. Absence of significance difference between sex groups may be due to an equal chance of exposure to the parasite (Tafese *et al*., 2012).

The mean PCV of parasitemic and aparasitemic cattle of the current study was almost similar. However, the PCV of cattle was significantly influenced by trypanosome infection in the previous study in the area (Denu *et al*., 2012). The lower PCV of apparently trypanosome free cattle could be due to various concurrent diseases and nutritional interference with development of anemia, conversely many cattle having high PCV also show to be infected in which it may be occurred due to early infection (Fantahun *et al*., 2012).

c) Entomological survey

During the current survey, tsetse flies were not captured. However, four genera of biting flies were captured. Previously, the current study area was infe - sted with *Glossina pallidipes, Stomoxys* and *Tabanus* (NTTICC, 2009; Denu *et al*., 2012), *Glossina morsitans submorsitans, Glossina fuscipes fuscipes, Haem - atopota* and *Chrysops* (Denu *et al.,* 2012). A total of 6236 biting flies were captured. From these, about 6133 (98.35%) were *Stomoxys* while the rest was tabanids which slightly agrees with previous report of 6.48% and 4.51% tabanids and 81.02% and 88.06% *Stomoxys* (muscids) during the late rainy and dry seasons, respectively (Denu *et al.,* 2012). Similarly, higher number both tsetse and biting flies were captured in non-controlled area than controlled area in Hawa Gelan district (Fantahun *et al*., 2012). The difference between the current and previous situation of tsetse flies might be attributed to implementation of tsetse flies control strategy undertaken in the district.

The mean apparent density of the captured biting flies of the current study was 86.61. The highest was recorded for *Stomoxys* (85.18 fly/trap/day) followed by *Haematopota* (0.97 fly/trap/day) while the lowest was recorded for *Chrysops* (0.19 fly/trap/day) followed by *Tabanus* (0.25 fly/trap/day). Denu *et al.* (2012) reported lower apparent density of muscids (*Stomoxys*) of 18.66 and 15.04 fly/trap/day in late rainy season and dry season, respectively; however, the apparent densities of tabanids (*Tabanus, Chrysops and Haematopota*) are comparable (1.49 and 0.77 fly/trap/day in late rainy and dry season, respectively).

In conclusion, the prevalence of the disease and its vectors, especially tsetse flies, were reduced when compared with situation of the area before five years. Implementation of integrated control strategy of the causal agent and its vectors were successfully resulted in reducing the occurrence of the disease. Thus, the integrated control strategy on application should be continued and disseminated to the

neighboring districts to avoid re-invasion of the area with tsetse flies.

V. Acknowledgement

The authors are grateful for financial support of Ambo University, and staffs of Ameya district Livestock Development and Animal Health Agency for their guidance and facilitating the field work. Finally, the farmers of the district are highly acknowledged for their support and encouragement during the work.

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Global Journal of Medical research: K Interdisciplinary
Volume 14 Issue 3 Version 1.0 Year 2014
Type: Double Blind Peer Reviewed International Research Journal
Publisher: Global Journals Inc. (USA)
Online ISSN: 2249-4618 & Print ISSN: 0975-5888

Sympathetic Ophthalmitis:Rare Possibility after an Uneventful Cataract Surgery

By Dr. Nimmi Rani
Arvind Eye Hospital, India

Abstract- Sympatheticophthalmitis is a rare, diffuse, bilateral, granulomatous, non-necrotising-panuveitis that develops after surgical or accidental trauma to one eye (exciting) followed by a latent period and the appearance of uveitis in the uninjured fellow eye (sympathizing). This case highlights the possiblerare occurrence of sympathetic ophthalmitis after an uneventful cataract surgery.

Keywords: sympathetic ophthalmitis, rare, uneventful, cataract surgery.

GJMR-K Classification: NLMC Code: WW 260

Strictly as per the compliance and regulations of:

Sympathetic Ophthalmitis:Rare Possibility after an Uneventful Cataract Surgery

Dr. Nimmi Rani

Abstract- **Sympatheticophthalmitis is a rare, diffuse, bilateral, granulomatous, non-necrotisingpanuveitis that develops after surgical or accidental trauma to one eye (exciting) followed by a latent period and the appearance of uveitis in the uninjured fellow eye (sympathizing). This case highlights the possiblerare occurrence of sympathetic ophthalmitis after an uneventful cataract surgery.**

Keywords: sympathetic ophthalmitis, rare, uneventful, cataract surgery.

I. Introduction

Our case report describes a rare presentation of a 45 yrs female developing sympathetic ophth - almitis after an uneventful cataract surgery. Sympathetic ophthalmitis is a bilateral, non-necrotising, granulomatous panuveitis developing after accidental or surgical trauma. William Mackenzie gave the clinical description and coined the term"sympathetic- coph - thalmitis".Ernst Fuchs in his publication in 1905 des - cribed -"Dalen Fuchs nodules".

II. Case Report

A 45years female, presented to us with the complaint of gradually progressive gross diminution of vision in both eyes for last two months. She had unde - rgone cataract surgery in her right eye in a camp three months back elsewhere. She had episodes of interm - ittent pain in both eyes in last 2 months with episodes of headache and tinnitus for the past one month. There was no relevant past history of trauma, recurrent ocular inflammation, joint pain or backache, tuberculosis, oral and genital ulcers, skin rash, episodes of diarrhea or any other symptom suggestive of collagen vascular disorders. No written record of her cataract surgery was available but according to the patient it was uneventful with good visual recovery in the immediate post operative period.

Visual acuity at presentation was HM and No PL and IOP was 13mm Hg and 70 mm Hg in the RE and LE respectively. Anterior segment examination of RE rev - ealed granulomatouskeratic precipitates on the corneal endothelium, koeppes nodules and neovascularization of iris. PCIOL was present. Anterior segment exam - ination of LE showed corneal edema, shallow anterior chamber with 360degrees posterior synechiae leading to seclusiopupillae and iris bombe with complicated cataract. Fundus examination of RE showed a normal disc and inferior exudative retinal detachment. Few dalenfuchs nodules were seen. LE funduscouldnot be visualized due to complicated cataract.

B-Scan of both eyes showed exudative retinal detachment with choroidal thickening. Laboratory investigations including mantoux test, serum ACE level, VDRL, antinuclear antibody tests,TLC, DLC, SGOT, SGPT were normal. CT scan of thorax was within normal limit. Fluorescein angiography of RE showed multiple hyperfluorescent leakage sites in the RPE during venous phase which persisted during late frames of study and pooling of dye in the areas of exudative retinal detac - hment.

Based on history,clinical features, B-scan and FFA findings diagnosis of sympathetic ophthalmitis was made. She was started on tab.methotrexate 20mg/ week, tab prednisolone (in tapering dose starting from 60mg/ day) with calcium and folate supplementation .Posterior sub-tenons injections of triamcinolone aceto - nide(0.5cc)was given both eyes. Steroid antibiotic eye drop was advised for both eyes and antiglauc -omae - yedrop was advised for left eye.

In subsequent follow-up, patient improved symptomatically with relief of pain and gradual resol - ution of anterior chamber reaction and vitritis in RE. LE did not show any evidence of improvement and deve - loped resistant secondary glaucoma with persistently high IOP (more than50 mm Hg) and became an absolute eye with nil visual potential.

On her last visit, after 10 months of above mentioned therapeutic regimen patient had visual acuity of HM in RE and LE showed no improvement. Bscan RE showed persistent vitritis with resolved retinal detachment. OCT RE showedepiretinal membrane with foveal atrophy.

III. Discussion

Incidence as reported in recent studies is 0.2% to 0.5% following injury and 0.01% following intraocular surgery. [1,2] Classically accidental trauma was the lea - ding cause but in the recent times surgery especially the vitreoretinal surgery seems to have surprassed it as the major inciting cause. Even though non-surgical trau -ma is considered the most common inciting cause for sympathetic ophthalmitis, there are reports of it deve - loping after vitreoretinal surgeries, [3] cataract surg -ery, [4]Yag-cyclodestruction, pars planaviterctomy, evis -

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ceration, Iridectomy, retinal detachment repair.[5,6] Cataract surgery that too uneventful has been reported very rarely as a precipitating event. In our case there was no history of trauma. Our patient had undergone only one intraocular surgery-cataract extraction with lens implantation with good visual gain in the immediate post-operative period. This suggests that this cataract surgery was the inciting event in our patient.

Latent period for the development of SO is variable with 1 month in 17%cases, 3 months in 50%cases and 1year in 90%of cases.[7]In our case, after a latent period of one month she started developing ocular symptoms.

Manifestations are usually asymmetric and have a wide spectrum starting with mild difficulty in near vision ,photophobia ,mild to severe granulomatous ant - erior uveitis with mutton fat keraticprecipitates, [8]iris thickening and elevated intraocular pressure (trab - eculitis)or hypotony(ciliary shutdown).Our patient prese - nted with defective vision in both the eyes. On exam - ination features of granulomatous anterior uveitis inclu - ding granulomatous keratic precipitates and koe -ppes nodules were found.IOP was also high.

Posterior segment examination often shows moderate to severe vitritis. Characteristic yellow-white mid-peripheral choroidal lesions-DALEN FUCHS nod - ules have been reported in addition to exudative retinal detachment and optic nerve swelling.[8,3] Compli - cations which worsen the prognosis are secondary glaucoma, complicated cataract and chronic macula - pathy leading to sub-retinal fibrosis and foveal atrophy. [9]Our patient had evident features of vitritis ,dalenfuchs nodules and exudative retinal detachment in both the eyes. In subsequent visits, our patient developed resis - tant secondary glaucoma and comp -licated cataract in the LE and foveal atrophy in the RE.

Extraocular manifestations include hearing - disturbance, alopecia, vitiligo and meningeal irritation.[4] Our patient also gave a vivid history of episodes of headache and tinnitus in the past one month.

Diagnosis depends mainly on history and clinical findings. Diagnostic modalities like B-scan, FFA , OCT and others only help us to rule out other diseases with similar clinical feature[2] B-scan and FFA in our patient revealed vitritis and exudative retinal detac - hment, supporting the diagnosis. OCT in our patient in the RE showed foveal atrophy explaining the persistent low vision in the RE.

Mainstay of treatment is immunomodulation using corticosteroid and other steroid-sparing immune - omodulators like cyclosporine, chlorambucil, azath - ioprine, cyclosporine and methotrexate.[10] Our patient was also treated with immunomodulators like pred - nisolone and methotrexate.

"Secondaryenucleation" –enucleation of the exciting eye after the development of sympathetic ophthalmitis is highly controversial. In our patient the exciting eye was the only seeing eye, so its preservation was crucial.

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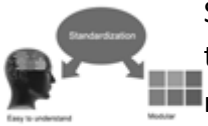

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Before start writing a good quality Computer Science Research Paper, let us first understand what is Computer Science Research Paper? So, Computer Science Research Paper is the paper which is written by professionals or scientists who are associated to Computer Science and Information Technology, or doing research study in these areas. If you are novel to this field then you can consult about this field from your supervisor or guide.

TECHNIQUES FOR WRITING A GOOD QUALITY RESEARCH PAPER:

1. Choosing the topic: In most cases, the topic is searched by the interest of author but it can be also suggested by the guides. You can have several topics and then you can judge that in which topic or subject you are finding yourself most comfortable. This can be done by asking several questions to yourself, like Will I be able to carry our search in this area? Will I find all necessary recourses to accomplish the search? Will I be able to find all information in this field area? If the answer of these types of questions will be "Yes" then you can choose that topic. In most of the cases, you may have to conduct the surveys and have to visit several places because this field is related to Computer Science and Information Technology. Also, you may have to do a lot of work to find all rise and falls regarding the various data of that subject. Sometimes, detailed information plays a vital role, instead of short information.

2. Evaluators are human: First thing to remember that evaluators are also human being. They are not only meant for rejecting a paper. They are here to evaluate your paper. So, present your Best.

3. Think Like Evaluators: If you are in a confusion or getting demotivated that your paper will be accepted by evaluators or not, then think and try to evaluate your paper like an Evaluator. Try to understand that what an evaluator wants in your research paper and automatically you will have your answer.

4. Make blueprints of paper: The outline is the plan or framework that will help you to arrange your thoughts. It will make your paper logical. But remember that all points of your outline must be related to the topic you have chosen.

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

6. Use of computer is recommended: As you are doing research in the field of Computer Science, then this point is quite obvious.

7. Use right software: Always use good quality software packages. If you are not capable to judge good software then you can lose quality of your paper unknowingly. There are various software programs available to help you, which you can get through Internet.

8. Use the Internet for help: An excellent start for your paper can be by using the Google. It is an excellent search engine, where you can have your doubts resolved. You may also read some answers for the frequent question how to write my research paper or find model research paper. From the internet library you can download books. If you have all required books make important reading selecting and analyzing the specified information. Then put together research paper sketch out.

9. Use and get big pictures: Always use encyclopedias, Wikipedia to get pictures so that you can go into the depth.

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

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

12. Make all efforts: Make all efforts to mention what you are going to write in your paper. That means always have a good start. Try to mention everything in introduction, that what is the need of a particular research paper. Polish your work by good skill of writing and always give an evaluator, what he wants.

13. Have backups: When you are going to do any important thing like making research paper, you should always have backup copies of it either in your computer or in paper. This will help you to not to lose any of your important.

14. Produce good diagrams of your own: Always try to include good charts or diagrams in your paper to improve quality. Using several and unnecessary diagrams will degrade the quality of your paper by creating "hotchpotch." So always, try to make and include those diagrams, which are made by your own to improve readability and understandability of your paper.

15. Use of direct quotes: When you do research relevant to literature, history or current affairs then use of quotes become essential but if study is relevant to science then use of quotes is not preferable.

16. Use proper verb tense: Use proper verb tenses in your paper. Use past tense, to present those events that happened. Use present tense to indicate events that are going on. Use future tense to indicate future happening events. Use of improper and wrong tenses will confuse the evaluator. Avoid the sentences that are incomplete.

17. Never use online paper: If you are getting any paper on Internet, then never use it as your research paper because it might be possible that evaluator has already seen it or maybe it is outdated version.

18. Pick a good study spot: To do your research studies always try to pick a spot, which is quiet. Every spot is not for studies. Spot that suits you choose it and proceed further.

19. Know what you know: Always try to know, what you know by making objectives. Else, you will be confused and cannot achieve your target.

20. Use good quality grammar: Always use a good quality grammar and use words that will throw positive impact on evaluator. Use of good quality grammar does not mean to use tough words, that for each word the evaluator has to go through dictionary. Do not start sentence with a conjunction. Do not fragment sentences. Eliminate one-word sentences. Ignore passive voice. Do not ever use a big word when a diminutive one would suffice. Verbs have to be in agreement with their subjects. Prepositions are not expressions to finish sentences with. It is incorrect to ever divide an infinitive. Avoid clichés like the disease. Also, always shun irritating alliteration. Use language that is simple and straight forward. put together a neat summary.

21. Arrangement of information: Each section of the main body should start with an opening sentence and there should be a changeover at the end of the section. Give only valid and powerful arguments to your topic. You may also maintain your arguments with records.

22. Never start in last minute: Always start at right time and give enough time to research work. Leaving everything to the last minute will degrade your paper and spoil your work.

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

24. Never copy others' work: Never copy others' work and give it your name because if evaluator has seen it anywhere you will be in trouble.

25. Take proper rest and food: No matter how many hours you spend for your research activity, if you are not taking care of your health then all your efforts will be in vain. For a quality research, study is must, and this can be done by taking proper rest and food.

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

27. Refresh your mind after intervals: Try to give rest to your mind by listening to soft music or by sleeping in intervals. This will also improve your memory.

28. Make colleagues: Always try to make colleagues. No matter how sharper or intelligent you are, if you make colleagues you can have several ideas, which will be helpful for your research.

29. Think technically: Always think technically. If anything happens, then search its reasons, its benefits, and demerits.

30. Think and then print: When you will go to print your paper, notice that tables are not be split, headings are not detached from their descriptions, and page sequence is maintained.

31. Adding unnecessary information: Do not add unnecessary information, like, I have used MS Excel to draw graph. Do not add irrelevant and inappropriate material. These all will create superfluous. Foreign terminology and phrases are not apropos. One should NEVER take a broad view. Analogy in script is like feathers on a snake. Not at all use a large word when a very small one would be sufficient. Use words properly, regardless of how others use them. Remove quotations. Puns are for kids, not grunt readers. Amplification is a billion times of inferior quality than sarcasm.

32. Never oversimplify everything: To add material in your research paper, never go for oversimplification. This will definitely irritate the evaluator. Be more or less specific. Also too, by no means, ever use rhythmic redundancies. Contractions aren't essential and shouldn't be there used. Comparisons are as terrible as clichés. Give up ampersands and abbreviations, and so on. Remove commas, that are, not necessary. Parenthetical words however should be together with this in commas. Understatement is all the time the complete best way to put onward earth-shaking thoughts. Give a detailed literary review.

33. Report concluded results: Use concluded results. From raw data, filter the results and then conclude your studies based on measurements and observations taken. Significant figures and appropriate number of decimal places should be used. Parenthetical remarks are prohibitive. Proofread carefully at final stage. In the end give outline to your arguments. Spot out perspectives of further study of this subject. Justify your conclusion by at the bottom of them with sufficient justifications and examples.

34. After conclusion: Once you have concluded your research, the next most important step is to present your findings. Presentation is extremely important as it is the definite medium though which your research is going to be in print to the rest of the crowd. Care should be taken to categorize your thoughts well and present them in a logical and neat manner. A good quality research paper format is essential because it serves to highlight your research paper and bring to light all necessary aspects in your research.

Informal Guidelines of Research Paper Writing

Key points to remember:

- Submit all work in its final form.
- Write your paper in the form, which is presented in the guidelines using the template.
- Please note the criterion for grading the final paper by peer-reviewers.

Final Points:

A purpose of organizing a research paper is to let people to interpret your effort selectively. The journal requires the following sections, submitted in the order listed, each section to start on a new page.

The introduction will be compiled from reference matter and will reflect the design processes or outline of basis that direct you to make study. As you will carry out the process of study, the method and process section will be constructed as like that. The result segment will show related statistics in nearly sequential order and will direct the reviewers next to the similar intellectual paths throughout the data that you took to carry out your study. The discussion section will provide understanding of the data and projections as to the implication of the results. The use of good quality references all through the paper will give the effort trustworthiness by representing an alertness of prior workings.

Writing a research paper is not an easy job no matter how trouble-free the actual research or concept. Practice, excellent preparation, and controlled record keeping are the only means to make straightforward the progression.

General style:

Specific editorial column necessities for compliance of a manuscript will always take over from directions in these general guidelines.

To make a paper clear

· Adhere to recommended page limits

Mistakes to evade

- Insertion a title at the foot of a page with the subsequent text on the next page
- Separating a table/chart or figure - impound each figure/table to a single page
- Submitting a manuscript with pages out of sequence

In every sections of your document

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· Keep on paying attention on the research topic of the paper

· Use paragraphs to split each significant point (excluding for the abstract)

· Align the primary line of each section

· Present your points in sound order

· Use present tense to report well accepted

· Use past tense to describe specific results

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· Shun use of extra pictures - include only those figures essential to presenting results

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

Abstract:

The summary should be two hundred words or less. It should briefly and clearly explain the key findings reported in the manuscript--must have precise statistics. It should not have abnormal acronyms or abbreviations. It should be logical in itself. Shun citing references at this point.

An abstract is a brief distinct paragraph summary of finished work or work in development. In a minute or less a reviewer can be taught the foundation behind the study, common approach to the problem, relevant results, and significant conclusions or new questions.

Write your summary when your paper is completed because how can you write the summary of anything which is not yet written? Wealth of terminology is very essential in abstract. Yet, use comprehensive sentences and do not let go readability for briefness. You can maintain it succinct by phrasing sentences so that they provide more than lone rationale. The author can at this moment go straight to shortening the outcome. Sum up the study, with the subsequent elements in any summary. Try to maintain the initial two items to no more than one ruling each.

- Reason of the study - theory, overall issue, purpose
- Fundamental goal
- To the point depiction of the research
- Consequences, including definite statistics - if the consequences are quantitative in nature, account quantitative data; results of any numerical analysis should be reported
- Significant conclusions or questions that track from the research(es)

Approach:

- Single section, and succinct
- As a outline of job done, it is always written in past tense
- A conceptual should situate on its own, and not submit to any other part of the paper such as a form or table
- Center on shortening results - bound background information to a verdict or two, if completely necessary
- What you account in an conceptual must be regular with what you reported in the manuscript
- Exact spelling, clearness of sentences and phrases, and appropriate reporting of quantities (proper units, important statistics) are just as significant in an abstract as they are anywhere else

Introduction:

The **Introduction** should "introduce" the manuscript. The reviewer should be presented with sufficient background information to be capable to comprehend and calculate the purpose of your study without having to submit to other works. The basis for the study should be offered. Give most important references but shun difficult to make a comprehensive appraisal of the topic. In the introduction, describe the problem visibly. If the problem is not acknowledged in a logical, reasonable way, the reviewer will have no attention in your result. Speak in common terms about techniques used to explain the problem, if needed, but do not present any particulars about the protocols here. Following approach can create a valuable beginning:

- Explain the value (significance) of the study
- Shield the model - why did you employ this particular system or method? What is its compensation? You strength remark on its appropriateness from a abstract point of vision as well as point out sensible reasons for using it.
- Present a justification. Status your particular theory (es) or aim(s), and describe the logic that led you to choose them.
- Very for a short time explain the tentative propose and how it skilled the declared objectives.

Approach:

- Use past tense except for when referring to recognized facts. After all, the manuscript will be submitted after the entire job is done.
- Sort out your thoughts; manufacture one key point with every section. If you make the four points listed above, you will need a least of four paragraphs.

- Present surroundings information only as desirable in order hold up a situation. The reviewer does not desire to read the whole thing you know about a topic.
- Shape the theory/purpose specifically - do not take a broad view.
- As always, give awareness to spelling, simplicity and correctness of sentences and phrases.

Procedures (Methods and Materials):

This part is supposed to be the easiest to carve if you have good skills. A sound written Procedures segment allows a capable scientist to replacement your results. Present precise information about your supplies. The suppliers and clarity of reagents can be helpful bits of information. Present methods in sequential order but linked methodologies can be grouped as a segment. Be concise when relating the protocols. Attempt for the least amount of information that would permit another capable scientist to spare your outcome but be cautious that vital information is integrated. The use of subheadings is suggested and ought to be synchronized with the results section. When a technique is used that has been well described in another object, mention the specific item describing a way but draw the basic principle while stating the situation. The purpose is to text all particular resources and broad procedures, so that another person may use some or all of the methods in one more study or referee the scientific value of your work. It is not to be a step by step report of the whole thing you did, nor is a methods section a set of orders.

Materials:

- Explain materials individually only if the study is so complex that it saves liberty this way.
- Embrace particular materials, and any tools or provisions that are not frequently found in laboratories.
- Do not take in frequently found.
- If use of a definite type of tools.
- Materials may be reported in a part section or else they may be recognized along with your measures.

Methods:

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

Approach:

- It is embarrassed or not possible to use vigorous voice when documenting methods with no using first person, which would focus the reviewer's interest on the researcher rather than the job. As a result when script up the methods most authors use third person passive voice.
- Use standard style in this and in every other part of the paper - avoid familiar lists, and use full sentences.

What to keep away from

- Resources and methods are not a set of information.
- Skip all descriptive information and surroundings - save it for the argument.
- Leave out information that is immaterial to a third party.

Results:

The principle of a results segment is to present and demonstrate your conclusion. Create this part a entirely objective details of the outcome, and save all understanding for the discussion.

The page length of this segment is set by the sum and types of data to be reported. Carry on to be to the point, by means of statistics and tables, if suitable, to present consequences most efficiently.You must obviously differentiate material that would usually be incorporated in a study editorial from any unprocessed data or additional appendix matter that would not be available. In fact, such matter should not be submitted at all except requested by the instructor.

Content

- Sum up your conclusion in text and demonstrate them, if suitable, with figures and tables.
- In manuscript, explain each of your consequences, point the reader to remarks that are most appropriate.
- Present a background, such as by describing the question that was addressed by creation an exacting study.
- Explain results of control experiments and comprise remarks that are not accessible in a prescribed figure or table, if appropriate.
- Examine your data, then prepare the analyzed (transformed) data in the form of a figure (graph), table, or in manuscript form.

What to stay away from

- Do not discuss or infer your outcome, report surroundings information, or try to explain anything.
- Not at all, take in raw data or intermediate calculations in a research manuscript.
- Do not present the similar data more than once.
- Manuscript should complement any figures or tables, not duplicate the identical information.
- Never confuse figures with tables - there is a difference.

Approach

- As forever, use past tense when you submit to your results, and put the whole thing in a reasonable order.
- Put figures and tables, appropriately numbered, in order at the end of the report
- If you desire, you may place your figures and tables properly within the text of your results part.

Figures and tables

- If you put figures and tables at the end of the details, make certain that they are visibly distinguished from any attach appendix materials, such as raw facts
- Despite of position, each figure must be numbered one after the other and complete with subtitle
- In spite of position, each table must be titled, numbered one after the other and complete with heading
- All figure and table must be adequately complete that it could situate on its own, divide from text

Discussion:

The Discussion is expected the trickiest segment to write and describe. A lot of papers submitted for journal are discarded based on problems with the Discussion. There is no head of state for how long a argument should be. Position your understanding of the outcome visibly to lead the reviewer through your conclusions, and then finish the paper with a summing up of the implication of the study. The purpose here is to offer an understanding of your results and hold up for all of your conclusions, using facts from your research and generally accepted information, if suitable. The implication of result should be visibly described. Infer your data in the conversation in suitable depth. This means that when you clarify an observable fact you must explain mechanisms that may account for the observation. If your results vary from your prospect, make clear why that may have happened. If your results agree, then explain the theory that the proof supported. It is never suitable to just state that the data approved with prospect, and let it drop at that.

- Make a decision if each premise is supported, discarded, or if you cannot make a conclusion with assurance. Do not just dismiss a study or part of a study as "uncertain."
- Research papers are not acknowledged if the work is imperfect. Draw what conclusions you can based upon the results that you have, and take care of the study as a finished work
- You may propose future guidelines, such as how the experiment might be personalized to accomplish a new idea.
- Give details all of your remarks as much as possible, focus on mechanisms.
- Make a decision if the tentative design sufficiently addressed the theory, and whether or not it was correctly restricted.
- Try to present substitute explanations if sensible alternatives be present.
- One research will not counter an overall question, so maintain the large picture in mind, where do you go next? The best studies unlock new avenues of study. What questions remain?
- Recommendations for detailed papers will offer supplementary suggestions.

Approach:

- When you refer to information, differentiate data generated by your own studies from available information
- Submit to work done by specific persons (including you) in past tense.
- Submit to generally acknowledged facts and main beliefs in present tense.

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

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ISSN 9755896