ORIGINAL ARTICLE

A Study of Estimation of Blood Levels of Lead by Atomic Absorption Spectrophotometer (AAS) in Fish Consuming Non-Vegetarian and Non-Fish Consuming Vegetarian Medical Students

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ABSTRACT

Background: Lead is in use since the pre-Christian era by humankind in various forms. Hence acute and chronic toxicity to humans due to lead salts also can be dated back to the pre-Christian era. With industrialization, the environmental pollution with metals like lead increased many folds. Contamination of marine sources is on raise due to the dumping of industrial wastes into rivers and oceans. This study is undertaken to estimate the effects on human beings due to environmental pollution by lead salts, more so on aquatic.

Aim: The objective of the study is to find out contamination of waters with lead causing toxicity to aquatic animals like fish. And consumption of such marine food effects in the Blood Lead Levels of human beings.

Methods: The objective achieved by selecting two groups of Medical students vegetarian (non-fish consuming) and fish consuming non-vegetarians and estimating their Blood Lead Levels by using an atomic absorption spectrophotometer (AAS).

Results: The average Blood Lead Levels are more in males than females (P-value 0.03) [15.99 ± 4.89; 10.9 ± 5.23).The Blood Lead Levels are slightly more in vegetarians than non-vegetarians (13.44 ± 5.57; 12.32 ± 6.93) but statistically not significant (P-value 0.577). Lead Levels are comparatively more in non-vegetarian males then vegetarian males (16.14 ± 5.68; 15.99 ±4.88).

Conclusion: The difference in the Blood Lead Levels of fish consuming non-vegetarians and non-fish consuming vegetarians is not statistically significant. The mean Blood Lead Levels of boys is more than girls. The mean Blood Lead Levels is higher when compared to developed countries standard.

INTRODUCTION

The lead is a highly lustrous, heavy, silvery-grey metal. It is soft and malleable and is abundant in soil, distributed throughout the earth's crust. It was one of the first metals known to man and widely used for domestic, industrial, and therapeutic purposes. [1] Some of the lead compounds include Lead acetate (sugar of lead) used in therapeutics, lead carbonate (white lead) used in paints, and lead oxide (litharge) for glazing of pottery, and enamelware. Lead tetroxide as vermilion (sindoor) applied by married Hindu women. Lead sulfide used as a collyrium (Surma)) for the eyes [1] Humans have used lead (Pb) for at least 7000 years because of its ease to extract and to work with and of widespread usage. It is highly malleable and ductile metal, as well as easy to smelt. In the early Bronze Age, lead used with antimony and arsenic. Lead's elemental symbol Pb, and is an abbreviation of its Latin name plumbum. Lead in lead compounds primarily exists in the divalent form. Metallic Lead (Pb^0) is resistant to corrosion. It can combine other metals to form various alloys. Pb4+ dominates organolead compounds. Organic lead compounds were used as gasoline additives in the past. Inorganic lead compounds used as pigments in paints, dyes, and ceramic glazes. Lead alloys used in batteries, shields from radiation, water pipes, and ammunition. The environmental lead came mainly from human activity and listed as a top toxic substance (ATSDR, 2005b). The phasing out of leaded gasoline, the removal of lead from paint, solder, and water supply pipes have significantly lowered blood lead levels (BLL) in the general population. But even today, Lead exposure in children remains a significant concern. Lead is not biodegradable, and ecotoxicity of Lead is increasing. For instance, the leaded fish sinkers or pellets lost in the bottom of lakes and river banks can be mistaken for stone and ingested by birds causing adverse effects including death (De Francisco et al.., 2003)[2].

The lead interferes with several aspects of cellular metabolism in tissues throughout the body, resulting in multisystemic effects. The pathophysiology of lead toxicity is complex and varies depending on the pattern of dose, the target organ, and the developmental stage of the subject. Many findings suggest that the multifaceted effects of lead on calcium metabolism play a fundamental role in the actions of Lead at a low dose. Lead can disturb calcium metabolism by substituting for this essential cation as an intracellular second messenger, or by altering the distribution of calcium in subcellular compartments. Protein kinase C, a calcium-dependent enzyme involved in the phosphorylation of regulatory proteins involved in multiple cellular processes, is activated by lead at intracellular concentrations as low as 10^{-10} M. Such concentrations may occur at blood lead concentrations of 5 to 10μg/dL. Lead binds more acidly than calcium to calmodulin, an essential calcium-binding regulatory protein. Lead inhibits Na^+, K^+-ATPase, which may, in turn, contribute to lead-associated increases in intracellular calcium. These and other effects of lead on cellular calcium biochemistry have substantial impacts on neurotransmission and vascular tone that, in turn, may contribute to lead-related health effects such as neurotoxicity and hypertension.[3]

The lead interferes with the biosynthesis of heme by inhibition of the enzyme delta-aminolevulinic acid dehydratase (ALAD). It also interferes with the mobilization and intracellular transfer of iron to protoporphyrin IX via a process other than in vivo inhibition of ferrochelatase. Erythrocyte protoporphyrins, such as zinc protoporphyrin, are increased as a result. [3] Lead poisoning is one of the significant environmental diseases among children in developing countries. A recent controversy involving a popular brand of noodles in India led to increased awareness about the potentials of lead toxicity. Extremely minimal amounts of lead exposure can have long-term and slowly accumulating harmful effects in children [4] Studies conducted to know the effects of exposure to chronic lead poisoning in adult as well as in children. The effects of increased blood levels in children resulting in both physical and psychological disorders, were well documented. [4, 5, 6] Studies were conducted in India and abroad on pollution of both drinking and seawater by lead as the industrial waste contaminates these water sources commonly.[7,8,9,10] Researchers also concentrated on the levels of lead in both aquatic plants and animals as consumption of their food products can cause chronic lead poisoning in human beings. [11, 12, 13] A pilot study conducted among medical students of Narayana Medical College involving 31 students between 19-21 years of age revealed that the blood levels of lead are more than 10ug/L in the majority of them [14]. These studies encouraged to do this study on the correlation of Blood Lead Levels among non fish consuming vegetarians and fish consuming non vegetarians to derive a conclusion concerning the contamination of marine waters with industrial wastage containing lead.

OBJECTIVES

1. The study objective is to find out contamination of waters with lead causing toxicity to aquatic animals like fish and consumption of such marine food effects in the Blood Lead Levels of human beings.
2. Along with these, the study is also focused on finding out area-specific mean Blood Lead Levels in Nellore, South Coastal district of Andhra Pradesh.
3. The mean Blood Lead Levels, thus obtained, were analyzed to derive a conclusion with the study objectives.

MATERIALS AND METHODS

The study included Forty medical students, twenty vegetarian non-fish consuming students, and twenty non-vegetarian fish consuming students. Twenty boys and twenty girls were selected ten in each group. The Blood samples from non-vegetarian fish consuming students were taken after giving them food containing about 250 Gms of fish. Vegetarian non-fish consuming students were given vegetarian food without fish. The Blood samples were taken between 6-12 hours after consuming the food. Informed consent was obtained from all the forty students of the study group. The subject's details were noted in the proforma. This study had the Institutional Ethical committee's clearance. 5ml of Whole Blood was collected from the study subjects in an EDTA coated vacutainer by venepuncture after taking all aseptic measures. The whole blood was stored at 4°C for further analysis. The estimation of Lead was carried out using Atomic Absorption Spectrophotometer (AAS) using flame emission technique. The whole blood was haemolysed by using precipitation solution, and the protein-free filtrate was obtained. The protein-free filtrate was used for the analysis of Lead. Analysis of whole blood for Lead was done with graphite furnace atomic absorption spectroscopy (GFAAS).

RESULTS

The results were analyzed by using students 'T' (Independent sample 'T' test) using IBM SPSS – 20.0 software and tabulated.

The results indicate that –

1. The average Blood Lead Levels are more in males than females (P-value 0.03) [15.99 ± 4.89; 10.9 ± 5.23) {Table 2}.
2. The Blood Lead Levels are slightly more in vegetarians than non-vegetarians (13.44 ± 5.57; 12.32 ± 6.93) but statistically not significant (P-value 0.577) {Table 1}.
3. The Lead Levels are, however, comparatively more in non-vegetarian males then vegetarian males (16.14 ± 5.68; 15.99 ±4.88) {Table 4}.

DISCUSSION

Lead and toxicity with lead are as old as human civilization. Due to industrialization and contamination with industrial wastes, lead pollution is on raise though specific measures like usage of unleaded petrol and other statutory the lead toxicity are still at alarming levels. The studies conducted by Pastorelli A, Baldini M, Stacchini P, Baldini G, Morelli S, Sagratella E et al. on sea products showed an increase in lead levels in certain fish products, indicating human exposure to lead toxicity due to their consumption. [11] Chaudhary S, Firdaus U, Ali S, Mahdi A found the prevalence of elevated BLL in children of Aligarh attending Pediatric OPD was about 44%. None of the child's habits or parental attributes were found to be related to the BLL in children. Only old and deteriorating paint on the house walls was significantly associated with elevated BLL. The flakes of old paints are likely to be chipped off from the wall and contaminate the house dust, which may be later ingested by the child through unhygienic eating habits. Could also explain the increased risk of elevated blood lead levels in younger children who exhibit more hand to mouth activities and less of hand hygiene. [4] Naicker N, Mathee A, Barnes B observed that Blood lead levels ranged from 0.8-32.3 μg/dl. The mean blood lead level in the total sample was 7.97μg/dl; 74% had blood lead levels ≥ 5μg/dl. The highest proportion (84%) of children with blood lead levels ≥ 5μg/dl was in Johannesburg. In the multivariate analysis, socioeconomic status has significantly associated with blood lead levels ≥ 5μg/dl. [6]

The studies conducted on Chinese population revealed that urban population had higher mean Blood Lead Levels and the Blood Lead Levels in males is more than females, studies also indicated that the mean value of Blood Lead Levels is higher in smokers and alcoholics. [15, 16] Singamsetty B, Gollapalli PK in their study found Mean blood lead levels of the workers were high among the workers working for longer duration in battery fitting unit. Toxicity signs and symptoms observed, and they were attributable to lead toxicity. Few cases had audiometric disturbances, and one had loss of vision. High levels of lead in blood were found to be related to hypertension and anemia among the workers. [17] Studies conducted on Indian population by Roy A, Bellinger D, Hu H, Schwartz J Ettinger A Wright, R et al. [18], and Parween A, Mohammad M, Upadhyay T, and Tripathi R[19] indicate that average blood lead level was more than 10µ mg/dl, similar results are observed in this study. The study by Hai D, Tung L, Van D, Binh T, Phuong H, Trung N, et al. showed that 80% (24/30) samples had PbS exceeding EPA form lead content in children's play area, and 79,49% of children living in Ban Thi Commune had BLL > 10μg/dL. They concluded that Lead mining and smelting activities have resulted in environmental pollution in the residential area near the mine. [5] Roy A, Bellinger D, Hu H, Schwartz J Ettinger A Wright, R et al. observed that Mean blood lead level was 11.4 ± 5.3 µg/dL. Blood lead was associated with higher anxiety (β = 0.27, p = 0.01), social problems (β = 0.20, p = 0.02), and higher scores in the ADHD (Attention Deficit Hyperactivity Disorder) index (β = 0.17; p = 0.05). The effect estimate was highest for global executive function (β = 0.42; p< 0.001). Higher blood lead levels in this population of young children are associated with increased risk of neurobehavioral deficits and ADHD, with executive function and attention being particularly vulnerable domains to the effects of lead. [18] Studies were also conducted to know the effect of regulations on lead usage on blood lead levels by WHO. [20, 21] A study on blood levels of refugee children was also carried out. [22]

CONCLUSION

The Blood Lead Levels of non-fish consuming vegetarians and fish consuming non-vegetarians analyzed after giving a fish meal of about 250gms to the fish consuming group. The blood sample is drawn and analyzed for Blood Lead Levels by using AAS and compared. The following conclusion is derived. There is no significant difference in the Blood Lead Levels of fish consuming non-vegetarians and non-fish consuming vegetarians. The mean Blood Lead Levels of boys is more than girls. The mean Blood Lead Levels is higher when compared to developed countries standard.

TABLES

Table-1 Average Blood Lead Levels in fish consuming Vegetarians and Non-Vegetarians Independent Samples T-Test (for Males and Females)

Group Statistics

	Type of Food	N	Mean	Std. Deviation	Std. Error Mean
Lead	Vegetarian	20	13.4450	5.57791	1.24726
	Non-Vegetarian	20	12.3250	6.93146	1.54992

Independent Samples Test

		Levene's Test for Equality of Variances		t-test for Equality of Means						
		F	Sig.	T	df	P Value	Mean Difference	Std. Error Difference	95% Confidence Interval of the Difference	
									Lower	Upper
LEAD	Equal variances assumed	1.239	.273	.563	38	577 NOT SIG	1.12000	1.98945	-2.90743	5.14743
	Equal variances not assumed			.563	36.337	.577	1.12000	1.98945	-2.91349	5.15349

Table -2 Average Blood Lead Levels in fish consuming Vegetarians (Independent Samples T-Test)

Group Statistics

	SEX	N	Mean	Std. Deviation	Std. Error Mean
LEAD	Males	10	15.9900	4.88909	1.54607
	Females	10	10.9000	5.23323	1.65489

Independent Samples Test

		Levene's Test for Equality of Variances		t-test for Equality of Means						
		F	Sig.	t	df	P VALUE	Mean Difference	Std. Error Difference	95% Confidence Interval of the Difference	
									Lower	Upper
LEAD	Equal variances assumed	.004	.949	2.248	18	.037 SIG	5.09000	2.26473	.33198	9.84802
	Equal variances not assumed			2.248	17.917	.037	5.09000	2.26473	.33041	9.84959

Table 3 Average Blood Lead Levels in fish consuming Non-Vegetarians (Independent Samples T-Test)

Group Statistics

	SEX	N	Mean	Std. Deviation	Std. Error Mean
LEAD	Males	10	16.1400	5.68393	1.79742
	Females	10	8.5100	6.06455	1.91778

Independent Samples Test

		Levene's Test for Equality of Variances		t-test for Equality of Means						
		F	Sig.	T	Df	P VALUE	Mean Difference	Std. Error Difference	95% Confidence Interval of the Difference	
									Lower	Upper
LEAD	Equal variances assumed	.048	.829	2.903	18	.009 SIG	7.63000	2.62842	2.10790	13.15210
	Equal variances not assumed			2.903	17.925	.010	7.63000	2.62842	2.10624	13.15376

Table 4 Average Blood Lead Levels in fish consuming Vegetarians and Non-Vegetarians in Males (Independent Samples T-Test)

Group Statistics

	Type of Food	N	Mean	Std. Deviation	Std. Error Mean
LEAD	Vegetarian	10	15.9900	4.88909	1.54607
	Non-Vegetarian	10	16.1400	5.68393	1.79742

Independent Samples Test

		Levene's Test for Equality of Variances		t-test for Equality of Means						
		F	Sig.	T	Df	P VALUE	Mean Difference	Std. Error Difference	95% Confidence Interval of the Difference	
									Lower	Upper
LEAD	Equal variances assumed	.329	.574	-.063	18	.950	-.15000	2.37087	-5.13102	4.83102
	Equal variances not assumed			-.063	17.606	.950	-.15000	2.37087	-5.13901	4.83901

Table 5 - Average Blood Lead Levels in fish consuming Vegetarians and Non-Vegetarians in Females (Independent Samples T-Test)

Group Statistics

	Type of Food	N	Mean	Std. Deviation	Std. Error Mean
LEAD	Vegetarian	10	10.9000	5.23323	1.65489
	Non-Vegetarian	10	8.5100	6.06455	1.91778

Independent Samples Test

		Levene's Test for Equality of Variances		t-test for Equality of Means						
		F	Sig.	t	Df	P VALUE	Mean Difference	Std. Error Difference	95% Confidence Interval of the Difference	
									Lower	Upper
LEAD	Equal variances assumed	.054	.819	.944	18	.358 NOT SIG	2.39000	2.53309	-2.93182	7.71182
	Equal variances not assumed			.944	17.622	.358	2.39000	2.53309	-2.94001	7.72001

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