ASSOCIATION OF BLOOD LEAD LEVELS AND WORKING MEMORY ABILITY OF PRIMARY SCHOOL CHILDREN IN RANAU, SABAH

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FACULTY OF MEDICINE AND HEALTH SCIENCES
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2015

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THESIS SUBMITTED IN FULFILLMENT FOR THE DEGREE OF MASTER OF SCIENCE (MEDICAL SCIENCE)

FACULTY OF MEDICINE AND HEALTH SCIENCES
UNIVERSITI MALAYSIA SABAH
2015

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ACKNOWLEDGEMENT

I wish to express my deepest gratitude and appreciation to my supervisor Associate Professor Dr. Shamsul Bahari b. Shamsuddin of the Faculty of Medicine and Health Sciences, Universiti Malaysia Sabah for his patient to advised and guided me throughout for the past few years. His encouragement provided me the necessary impetus and support to complete the research and publish this thesis.

My appreciation also dedicated to the Dean of School of Medicine and Post Graduate Centre of UMS for organising this excellents programme. To The Director of Sabah Health Department, Director of Sabah Education Department, Head Masters and Health Teachers of S.K Poring, S.K. Bongkud, S.K Lohan, S.K Ulu Sipitang and S.K Dalas for the cooperations. To Matron Hasnah of Ranau Health Centre and School Health Team of KKIA Sipitang and KKIA Pitas who has been helping me in most of the sampling, testing of Working Memory ability, distributing and collecting of questionaires.

My special sense of gratitude and appreciation also the to the Dean of Faculty of Food and Nutrition, Universiti Malaysia Sabah, Associate Professor Dr. Sarifudin Md. Shaarani for allowing me to use the laboratory facility, also to Mr. Wilter Kisin, the Laboratory Assistant, for teaching and assisting me in laboratory procedures and to Mr. Michael Joseph of Sabah Health Department for english editing work.

My special thanks also to the Ministry Science, Technology and Innovation for providing the grant of FRGO280-SKK-2/2010 which has been very useful to purchase standards, reagent and disposable material. My appreciation also goes to Jabatan Perkhidmatan Awam through Ministry of Health, for providing the study fee support under the Federal Government Scholarship Award.

My sense of happiness also shared with my family especially my beloved wife Dg. Tuah @ Rosnah bt. Samah, for her patient and encouragement, to my sons Uwais Muizuddin Arshad, Uzair Aminuddin Arshad and Ubaidullah Arshad for their continuous support and to my fellow colleague Mr. Sundusin Hj. Nganro for being a good friend during our hard time. Finally, to those involved directly or indirectly to this research. May Allah bless you alls.

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ABSTRACT

A cross sectional study on the association of Blood Lead Levels (BLL) and Working Memory (WM) ability of primary school children in Ranau, Sabah was conducted from September 2012 till October 2013 . One hundred schools children were selected from 5 schools which consisted of three from Ranau (Study Population, n=50), one each from Pitas and Sipitang (Control Population, n=50). The objectives were to study the association of BLL and WM ability of Primary School Children in Ranau Sabah, to identify the difference in BLL and WM Ability between sex and type of populations, the correlation between respondent background and BLL's, to determine the level of WM ability and the correlation of BLL and WM. Blood samples were taken by venous blood draw using disposable syringes and collected in plastic sterile tube mixed with K_2EDTA. The samples were maintained at 4ºC and transported to Universiti Malaysia Sabah laboratory. BLL was tested using Inductively Coupled Plasma Mass Spectrometry (ICP-MS) Perkin-Elmer. WM ability was measured by extraction of McCarthy Intelligent Quotient (IQ) Index Test and known as WM Index Cummulative Score (WMICS). Demographic background obtained from the distributed questionnaires. One sample T Test show, p-value > 0.05 for BLL at 4.90 µg/dL - 5.28 µg/dL lower than BLL for 'action level' at 10 µg/dL (ATSDR, 2007). The independent Samples T test of mean show BLL was higher in boys compared to girls ($p < 0.05$), no significant difference between the study population and control population ($p > 0.05$). ANOVA tests for BLL and race/ethnicity ($p > 0.05$), BLL and mode of transportation to school ($p > 0.05$), BLL and religion ($p > 0.05$), BLL and education level ($p > 0.05$). The WM ability of school children was high, 76% scored 78% -100% of WMICS. Mann Whitney U Test show type of populations influenced the WMICS ($p < 0.05$) but not gender ($p > 0.05$). Pearson Correlation Test show a negative association for BLL and WMICS ($r= -0.621$) at medium level where $r = (-0.51) – (-0.70)$). The research result was a reinstatement of negative associations of BLL and WM ability among school children. However, since the study results are not considered representing the whole problem of BLL of school children in Sabah. Further research is needed such as to carry out a comprehensive and complete lead study and monitoring programme which involves all areas and parameters, such as age, environmental samples, biomarkers, nutritional factors, KAP's and complete IQ test together with continuous health promotion and education programmes with inter-agency collaboration. Such comprehensive programme will ensure the lead elimination programme become a reality in Sabah.

ABSTRAK

HUBUNGKAIT DI ANTARA PARAS PLUMBUM DALAM DARAH DAN KEUPAYAAN MEMORI KERJA DI KALANGAN MURID-MURID SEKOLAH DI DAERAH RANAU, SABAH

Satu kajian Hirisan Lintang ke atas hubungkait di antara Paras Plumbum Dalam Darah (BLL) dan keupayaan Memori Kerja (WM) di kalangan murid-murid sekolah di Ranau, Sabah. Telah dilaksanakan pada bulan September 2012 sehingga bulan Oktober 2013. Seramai seratus orang murid telah dipilih daripada 5 buah sekolah iaitu 3 dari Ranau (Populasi Kajian, n=50), dan setiap satu daripada Pitas dan Sipitang (Populasi Kawalan, n=50). Objektif kajian adalah untuk menentukan hubungkait BLL dan WM di kalangan murid sekolah di Ranau, Sabah, mengenalpasti perbezaan BLL dan WM terhadap perbezaan jantina dan jenis populasi, korelasi latarbelakang responden dan BLL, menentukan paras BLL dan keupayaan WM serta korelasi di antara BLL dan WM. Sampel darah diambil daripada vein dengan menggunakan jarum pakaibuang dan dimasukkan ke dalam tiub plastik steril yang mengandungi K_2EDTA. Suhu sampel dikekalkan pada paras 4ºC dan diangkut ke makmal Universiti Malaysia Sabah. BLL diuji dengan menggunakan mesin Inductively Coupled Plasma Mass Spectrometry (ICP-MS) Perkin-Elmer. Keupayaan WM diukur dengan menggunakan ekstrak soalan Indeks IQ McCarthy dan dikenali sebagai Skor Kumulatif Indeks Memori Kerja (WMICS). Manakala, latarbelakang demografi diperolehi daripada borang soalselidik yang diedarkan. Ujian T Satu Sampel menunjukkan, nilai $p > 0.05$ dimana BLL adalah pada paras 4.90 µg/dL - 5.28 µg/dL iaitu kurang daripada 'paras bertindak' pada 10 µg/dL (ATSDR, 2007). Ujian T sampel bebas menunjukkan min BLL bagi murid lelaki lebih tinggi berbanding murid perempuan ($p < 0.05$) dan tiada perbezaan signifikan di antara populasi kawalan dan populasi kajian ($p>0.05$). Hasil ujian ANOVA ke atas BLL dan jenis bangsa ($p > 0.05$), BLL dan jenis pengangkutan ke sekolah ($p >0.05$), BLL dan agama ($p > 0.05$), BLL dan tahap pendidikan ($p > 0.05$). Keputusan kajian mendapati keupayaan WM adalah tinggi. Dimana seramai 76% responden memperolehi skor WMICS pada julat 78% - 100%. Ujian Mann Whitney U menunjukkan jenis populasi mempengaruhi skor WMICS ($p < 0.05$) tetapi tidak bagi jenis jantina ($p > 0.05$). Keputusan ujian korelasi Pearson menunjukkan hubungkait negatif diantara BLL dan WMICS ($r= -0.621$) iaitu pada paras sederhana di antara nilai $r = (-0.51) – (-0.70)$. Keputusan kajian ini merupakan pernyataan semula tentang hubungkait negatif di antara BLL dan keupayaan WM di kalangan murid sekolah. Walaubagaimanapun, kajian ini tidaklah boleh dianggap mewakili keseluruhan masalah BLL di negeri Sabah sepenuhnya. Oleh itu, satu kajian yang lengkap dan komprehensif serta melibatkan program pengawasan yang meliputi semua bidang dan parameter seperti umur, sampel persekitaran, lain-lain penanda biologi, faktor pemakanan, KAP dan ujian IQ yang lengkap berserta dengan aktiviti promosi kesihatan yang berterusan beserta dengan kerjasama dengan lain-lain agensi perlu dilaksanakan pada masa akan datang. Program komprehensif dan lengkap seperti ini diharap akan memastikan eliminasi pencemaran plumbum akan menjadi realiti di Sabah.

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LIST OF ABBREBIATION

µg/dL	Micro gram per decilitre
ADHD	Attention deficit/hyperactive disorder
ALAD	δ- Aminolevulinic Acid Dehidrogenase
ALAS	δ- Aminolevulinic Acid Synthase
AMPA	α- Amino-3-hydroxy-5-methyl-isoxazolepropionic acid
ATP	Adenosine Triphospate
ATSDR	American Toxicology Safety Datasheet Registry
BBB	Blood Brain Barrier
BLL	Blood Lead Level
BTB	Blood Testis barrier
Ca	Calcium
CA1	Cornu Ammonis area 1
CA3	Cornu Ammonis area 3
CaMKII	Calmadoulin-dependant protein kinase II
CANTAB	Cambridge Neuropsychological test Automated Battery
CDC	Center of Disease Control
CNS	Central Nervous System
DG	Dentate gyrus
DMSA	Dimercaptosuccinic acid
EC	Entorhinal cortex
ESP	Excitory Post-sinapse polarisation
GFAP	Gene Fibliary Acidic Protein
ICP-MS	Inductively Coupled Plasma Mass Spectrometry
ILO	International Labour Organisation
IQ	Intelligent Quotient
K	Kalium
KAP	Knowledge, Attitude and Practice
LTM	Long Term Memory
LTP	Long Term Potentiation
Mg	Magnesium

mg/kg	Miligram per Kilogram
mg/l	Miligram per litre
MTL	Media Temporal Lobe
Na	Natrium
NGO	Non Government Organisation
NHANES	National Health and Nutrition Examination Survey
NHMRC	National Health Morbidity Research Centre
NMDAR	N-metil-D-aspartate Receptor
NO	Nitric Oxide
UMS	Universiti Malaysia Sabah
Pb	Plumbum (Lead)
PbBPs	Lead Binding Proteins
PBG	Porphobilinogen
PFC	Prefrontal Cortex
PKC	Protein Kinase C
PMCA	Plasmic Membrane Casium ATPase
PPB	Part Per Billion
PPM	Part Per Million
PRN	Pusat Racun Negara
RBC	Red Blood Cell
Rh	Rodium
RNA	Ribonucleic Acid
SPSS	Statistical package for Sosial Science
STM	Short Term Memory
VGCC	Voltage Gated Calcium Channel
WHO	World Health Organisation
WISC	Weischler Intelligent Score
WM	Working Memory
WMC	Working Memory Capacity
WMI	Working Memory Index
WTO	World Trade Organisation
ZPP	Zink Protoporphyrin Acid

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CHAPTER 1

INTRODUCTION

1.1 Introduction

Lead is a highly toxic metal with bluish grey colour. It exists naturally in the earth's crust around 15-20 mg/kg. It's a permeable, soft and not an efficient electricity conductor. Corrosion resistant and physically affected if exposed to the air for a long period of time. Coexist and mined with the other metal such as zinc, silver and copper. Naturally mined in the form of galena (PbS), cerrussite ($PbSO_3$) and anglesite ($PbCO_4$). Lead has been mined, commercially and domestically used for thousands of years (ATSDR, 2007).

Lead is classified as a heavy metal for its permeability and corrosion characteristic. The utilisation of lead has been tremendously increased as an anti-knock function for fuel of gasoline engine. Consequently lead has become one of the major contributors for atmospheric pollution globally. During 1920's till 1950's lead were also used as a glazing material for paint and contributed to contamination and lead poisoning incidents especially in developed countries (Hernberg, 2000)

According to El-Ansary *et al* (2011), the mechanism of lead poisoning is due to itsability to mimic the function of calcium ion which plays a significant role in many biochemical processes in living organism. Lead has the ability to compete and substitute calcium in the process of where calcium is needed. This situation can cause disturbance to the cell function, imbalance of calcium homeostasis and combined to sulfhydryl group. Lead can also cause the released of calcium ion from the mitochondria and causing damage to the mitochondria itself and its membrane. Lead also has ability to substitute zinc in zinc mediated biochemical process, increase oxidative pressure and finally alter the process of lipid metabolism (El-Ansary *et al.,* 2011).

According to Ahamed and Siddiqui (2007), toxicity effect of lead will lead to abnormality in myelin formation, reduced neurotransmitter density and number of neurotransmitter released. It can cause disturbance in information transmission in the brain. Lead can increase lipid peroxidation activities, inhibits haem biosynthesis process which will cause anaemia, reduce the cellular metabolism activities and affects cellular apoptosis process. All of these toxicity effects will finally influenced the natural metabolism process and affect the process of transmission of information. Finally, lead can cause disturbance to cellular energy formation process which is vital for living organism (Ahamed and Siddiqui, 2007).

According to Liu *et al* (2013), the main mechanism of lead upon neurological system is to increase the affinity towards the Ca^{2+} binding site and cause disturbance in Ca^{2+} metabolism process. Lead has an ability to substitute Ca^{2+} in Ca/Na ATP Pump, affect the neuron and caused malformation to the inter cell connection. Lead affects the neurotransmitters such as glutamate, acetylcholine and GABA which is vital in the process of storing and recall of memory. Lead also activates the Protein Kinase C which plays a role as an information transmission inhibitor and can cause disturbance to Ca^{2+} mediated apoptosis process. The most affected brain region is hippocampus, which plays a significant role in the process of Working Memory (WM), Short Term Memory (STM), Long Term Potentiation (LTP), auto association and recall of Long Term Memory (LTM) process. Prefrontal Cortex region (PFC) where Working Memory (WM) and Long Term Memory (LTM) process take place is also affected. Consequently, the disturbance of these processes will affect the whole neurological system and finally will cause the reduction of IQ amongst those who get lead poisoning (Sanders *et al.,* 2009; Liu *et al.,* 2013).

Generally, the occurrence of lead exposure is continuous and prolonged. The exposure starts since the early years of childhood development. Lead exposure among children will cause disturbance to neuron migration process, cellular proliferation process and brain synaptic activities. The most vulnerable and affected group are foetus and children under 4 years old of age. The symptoms such as reduction in IQ level, abnormal behaviour such as hyperactive, lack of

concentration, hearing problem and poor academic performance were detected amongst this group (Naicker *et al.,* 2010).

The vulnerability of children to lead poisoning is due to their anatomical and physiological condition. Lead absorptions rate for children is 40%-50% higher compared to adult population at 3% - 10% (ATSDR, 2007). The children's Central Nervous System (CNS) is immature and still developing. The signs and symptoms of lead poisoning are easily detectable and irreversible. Lead poisoning at high dosage will cause seizure, coma and death. The issue of lead poisoning and it's correlation to Working Memory (WM) has become one of the most discussed topics among scientist worldwide lately (Llop *et al.,* 2013).

Lead poisoning is still one of the major health problems among children and certain communities in particular a country. The problems are contributed by the community background such as poverty, urbanisation, abandoned group and neglected societies. Other problems are due to the types of occupation such as living in a mining area and bring back poison issue (Mahram *et al*, 2007: Moodie *et al.,* 2010). In Malaysia, previous study revealed that most of the lead pollution was due to the heavy traffic, urbanisation development and industrialisation. All these factors have immensely contributed to the release of lead into the atmosphere (Han *et al.,* 2012). Though, lead poisoning is highly influenced by demographical background of the exposed population (Shamsul *et al.,* 2005).

Primary School children were chosen as a study population due to their vulnerability and exposure to lead poisoning compared to the adult population. In fact, 99% of lead absorbed by adult will be removed from their body but only 32% for children (Shamsul B.S. 1998). These conditions also worsen because children are more active and absorb more lead from the environment. Children are vulnerable to malnutrition due to their high metabolic rate which needs such level of metabolism to support their development and growth process. Insufficient amount of calcium and iron in children's diet will cause lead absorption into the body system (Mathee *et al.,* 2013). Finally, the selection of the Primary School for

this research was due to the fact that, this is the only education institution available for 7–12 years old age of children in Malaysia (Jabatan Pelajaran Sabah, 2010).

Ranau District is situated in the interior part of Sabah. Link to the Kota Kinabalu City about 100 km by fully paved road. Ranau is a mountainous and hilly region with the altitude of 1000-2000 meters at the Crocker Range (Pejabat Daerah Ranau, 2010). Mount Kinabalu, the highest mountain in South East Asia is located here. Ranau was formerly the main producer of copper in Malaysia. Copper was mined in Mamut Copper mine, opened during the early 70's. It ceased operation in the early 2001's due to environmental and health concerned. Open mined method was used which covered an area of 20,000 acres (Jopony *et al.,* 2009). The mine was in operation for almost 20 years. The Mamut Copper mine has been the single main contributor of the environmental and atmospheric pollution in this region. The Ranau valley is surrounded by Crocker Range. The geographical conditions of the valley contribute to the atmospheric movement concentrating in that region. The dust and pollutant produced by the mining activities accumulated and polluted rivers, streams and water supply in this region. This kind of situation has exposed the local population to pollutant especially lead through consumption of water, food and air. This was proven through research carried out by Pusat Racun Negara and Yayasan Sabah (Pusat Racun Negara, 1997).

1.2 Problem Statement

Lead is categorised as heavy metal which is bendable and anti-crust. Lead has been used by human for thousands of years. The anti-knock function and additive in petrol has tremendously increased the utilisation of lead in gasoline worldwide. Lead is known to be one of the environmental health and children related issue in the modern world. Scientific society has considered lead poisoning among children as a public health disaster worldwide (ATSDR, 2007).

Currently, the subject of lead poisoning has become one of the important topics discussed and studied for public health field of study in developed countries. Early exposure of lead can cause neuron disturbance in neuron migration process, cellular proliferation process and synapse development. The most vulnerable group

are children aged between 2–4 years and foetus. The effect could be IQ reduction, hearing loss and hyperactive, loss of concentration and poor academic performances (Wang *et al.,* 2009). Later stage of lead health effect could be of delayed puberty, high blood pressure and infertility (Naicker *et al.,* 2010). Hence, some of the researchers have suggested that there is no threshold limit for lead exposure and the health effect is irreversible (Jedrychowski *et al.,* 2009).

Working memory is one of the key components of intelligence and can be part of the IQ test. The level of working memory can be a good predictor to the level of IQ among children. Working memory ability is one of the significant factors for learning and making decision processes among children. According to Alloway and Passolunghi (2011), the low level of Working Memory ability is an indicator for poor performance of learning skill especially arithmetic and mathematic. Since the Working Memory is part of the whole of IQ of the individuals. The high level of BLL will ultimately affecting the Working Memory ability if individuals (Alloway and Passolunghi, 2011).

Lead poisoning related field of study in developed countries is more advanced compared to developing countries. In Malaysia lead related studies considered as a step behind and not being highlighted in scientific research (Shamsul, B.S. 1998). Though, there were a few research conducted in Malaysia such as high blood lead level of students in Klang Valley (Shamsul, B.S. 1998), High Blood Lead Level of students in the West Coast of Sabah (Pusat Racun Negara and Yayasan Sabah, 1998) and Blood Lead Level < 10 µg of students in Terengganu (Shamsul, 1998). The novelty of this research is to provide a current level of BLL among schools children in Ranau, Sabah after more than a decade since the issue of high BLL in this area being highlighted in 2001. This research would also prove if the government decision to cease the operation of Mamut Copper mine was the right decision or contrary.

Blood Lead Level Surveillance programme has not been carried out in our country compared to developed countries. As a result, there is no baseline data for Blood Lead level (BLL) in our population particularly in children. The lack of such

programme has contributed to the absence of data on lead contributing factors, lead background and records of lead poisoning in our country (Shamsul, 1998).

The level of awareness in children related to lead poisoning among public are also limited and at minimum level. This situation can be seen in government agencies and department such as Health Department, Education Department, Human Resource Department, Welfare Department and Local Government. As such, it has resulted in the lack of inter agencies programmes conducted in our state. Especially involving the related government agencies and NGO's to tackle the issue of lead poisoning among children particularly among school children. Though, Malaysia has joined the global effort to ban the usage of leaded gasoline in 1996. But, we still need a clear and organised programme to monitor the level of lead in the blood amongst the population of Malaysia (Shamsul *et al.,* 2005). Thus, there is a need for us to have a BLL Surveillance Programme in our country which has been proven successful in a few developed countries such a United State and Japan (Wijngaarden *et al.,* 2011).

1.3 Research Objectives

a. To identify Blood Lead Level (BLL) of Primary School Children in Ranau District and the differences of BLL between boys and girls

b. To identify the different of BLL between study Population and Control Population.

c. To identify the correlation of respondents background and the BLL of school children.

d. To identify the level of Working Memory ability of Primary School Children of Ranau District.

e. To identify the correlation of BLL and Working Memory ability of Primary School Children in Ranau district.