2020-04

Exploring the relationship between diabetes mellitus and environmental fluoride contamination in selected areas of Tanzania

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EXPLORING THE RELATIONSHIP BETWEEN DIABETES MELLITUS AND ENVIRONMENTAL FLUORIDE CONTAMINATION IN SELECTED AREAS OF TANZANIA

Epafra Godson

A Dissertation Submitted in Partial Fulfilment of the Requirements for the Master`s in Life Sciences of the Nelson Mandela African Institution of Science and Technology

Arusha, Tanzania

April, 2020
ABSTRACT

Prevalence of Diabetes Mellitus in Tanzania is on the increase. Some few studies have linked fluoride contamination with possibilities of having effects on the insulin production resulting to Diabetes Mellitus. This has not been fully explored in Tanzania. Therefore, this study was undertaken to examine any relationship between fluoride levels in human blood serum and Diabetes Mellitus status of individuals in specific two wards of Tanzania. Cross sectional survey was conducted to explore the relationship between Diabetes Mellitus and fluoride in human blood serum whereby, two locations with different characteristics of environmental fluoride contamination in water sources were investigated. These areas were Ngarenanyuki ward (fluoride endemic area) in Arusha region and Mlandizi ward (non-endemic area) in Pwani region. The survey was conducted where 300 individuals (participants) were sampled from the study locations and consent for fasting blood sugar levels test, whereby, 97 individuals among them consent for both fasting blood sugar test and fluoride levels in blood serum determination. The prevalence of Diabetes Mellitus through fasting blood sugar test was 8.3%. High Diabetes prevalence was recorded at Mlandizi ward compared to Ngarenanyuki ward, though the difference was not significantly different (p = 0.144). Serum fluoride > 0.06 mg/l was significantly high in Ngarenanyuki (79%) compared to Mlandizi (21%) (P < 0.001). However, the current study did not establish any association between Fasting Blood Sugar tests and fluoride levels in blood serum (Pearson`s correlation coefficient analysis, r = - 0.0632; P < 0.663). Further work to include other cofounders is therefore recommended in order to understand broadly the influence of fluoride in Diabetes prevalence in fluoride endemic areas.
DECLARATION

I, Godson Epafra do hereby declare to the Senate of Nelson Mandela African Institution of Science and Technology that this dissertation is my original work and that it has neither been submitted nor being concurrently submitted for degree award in any other institution.

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Dr. Revocatus L. Machunda......................................................................

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CERTIFICATION

The undersigned certify that, have read the dissertation titled “Exploring the Relationship between Diabetes Mellitus and Environmental Fluoride Contamination in Selected Areas of Tanzania” and recommend for examination in fulfillment of the requirements for the degree of Master of Life Sciences of the Nelson Mandela African Institution of Science and Technology.

Dr. Gabriel M. Shirima
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Date

Dr. Revocatus L. Machunda
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Date
ACKNOWLEDGEMENTS

First, foremost and above all, I praise the Lord, the Almighty God for answering my prayers by giving me the strength to continue with this work up to this stage.

I would like to express my sincere gratitude to my supervisors; Dr. Gabriel M. Shirima and Dr. Revocatus Machunda, both - Senior Lecturers at the Nelson Mandela African Institution of Science and Technology (NM-AIST), for their constructive guidance, valuable support and encouragement throughout the whole process of undertaking this research.

I am thankful to the African Development Bank and FLOWERED project based at Nelson Mandela African Institution of Science and Technology for financing research activities as well as technical support. I wish to thank Arusha Technical College (ATC) for granting permission during the study period.

I am also, grateful to Mr. Frank Olotu for assisting in data collection and Ward Executive Officers (WEO) in Ngarenanyuki and Mlandizi wards, for allowing me to conduct this research in their administrative areas and all participants in this research who took time out of their busy schedules to participate in the study.

I further wish to express my thanks and appreciation to the Nelson Mandela African Institution of Science and Technology (NM-AIST) and particularly School of Life Sciences and Bioengineering (LiSBE), during my study period. They have provided me skills, guidance and good academic environment.

Finally, special thanks to my family, my wife Upendo Mushi, my sons and daughter, Livingstone, Liberty and Esther for their understanding and encouragement. Without their support, I would never had the energy to complete this work.
DEDICATION

I dedicate this work to my parent, Mama Eshikensia Robert and to my beloved wife, Upendo Mushi, my sons and daughter, Livingstone, Liberty and Esther for their love and patience throughout the stages of development of this research work.
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<th>Abbreviation</th>
<th>Full Form</th>
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<tr>
<td>CHW</td>
<td>Community Health Workers</td>
</tr>
<tr>
<td>DM</td>
<td>Diabetes Mellitus</td>
</tr>
<tr>
<td>FBS</td>
<td>Fasting Blood Sugar</td>
</tr>
<tr>
<td>F</td>
<td>Fluoride</td>
</tr>
<tr>
<td>Mg/L</td>
<td>Milligram per liter</td>
</tr>
<tr>
<td>Mmol/L</td>
<td>Millimoles per liter</td>
</tr>
<tr>
<td>mRNA</td>
<td>Messenger ribonucleic acid</td>
</tr>
<tr>
<td>M Sc</td>
<td>Masters in Science</td>
</tr>
<tr>
<td>MoH</td>
<td>Ministry of Health</td>
</tr>
<tr>
<td>NIMR</td>
<td>National Institute for Medical Research</td>
</tr>
<tr>
<td>NM- AIST</td>
<td>Nelson Mandela African Institution of Science and Technology</td>
</tr>
<tr>
<td>PI</td>
<td>Principal Investigator</td>
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<tr>
<td>Q</td>
<td>Quintile</td>
</tr>
<tr>
<td>RPM</td>
<td>Revolution per minute</td>
</tr>
<tr>
<td>SPSS</td>
<td>Statistical package for social sciences</td>
</tr>
<tr>
<td>TISAB</td>
<td>Total Ionic Strength Adjustment Buffer</td>
</tr>
<tr>
<td>UN</td>
<td>United Nation</td>
</tr>
<tr>
<td>WEO</td>
<td>Ward Executive Office</td>
</tr>
<tr>
<td>WHO</td>
<td>World Health Organization</td>
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CHAPTER ONE

INTRODUCTION

1.1 Background of the problem

The term Diabetes Mellitus, describes a metabolic disorder of multiple aetiology characterized by chronic hyperglycaemia with disturbances of carbohydrate, fat and protein metabolism, resulting from defects in insulin secretion, insulin action, or both (Rambhade et al., 2010). Diabetes Mellitus is a serious public health concern in Tanzania (Mwangome et al., 2018). The global Diabetes prevalence in 2019 is estimated to be 9.3% (463 million people), and is expected to rise up to 10.2% (578 million) by 2030 and 10.9% (700 million) by 2045, prevalence is higher in urban (10.8%) than rural (7.2%) areas (Saeedi et al., 2019). Worldwide, the prevalence of Diabetes Mellitus in adults aged 18–99 years was estimated to be 8.4% in 2017 and predicted to rise to 9.9% in 2045 (Cho et al., 2018). These sharp rises of Diabetes health problem have forced governments worldwide to establish policies and guidelines to ameliorate health and economic impact of Diabetes Mellitus (Saeedi et al., 2019). Most of interventions in preventing Diabetes Mellitus in Tanzania are centered on lifestyle, diet, lack of physical activity, hereditary and autoimmune as major contributing factors in addressing the problem. Meanwhile, there are other factors that can cause Diabetes Mellitus, including excessive consumption of fluoride, resulting into reduction of insulin production (Mithal, 1993; Pain, 2015). Similar studies indicated that consumption of water with high level of fluoride resulting into hyperglycemia due to diabetogenic effect of fluoride deriving from inhibition of key enzymes in glycolysis and in the Krebs cycle (Grucka-Mamczar et al., 2007).

Fluoride is an electronegative charged inorganic form of fluorine, naturally capable of reacting with other elements, with multiple implications for human and animal health (Martinez-mier, 2011). Presence of high levels of fluoride in some regions of Tanzania is associated with volcanic activities in the African Rift Valleys (“The effects of fluoride on human health in Eastern Rift Valley, northern Tanzania,” 2013). Fluoride distribution is not uniform throughout the country as there are some regions exhibiting higher levels of fluoride in water above the recommended values of 1.5 mg/l and tolerable upper of 4 mg/l for human consumption purposes (Tanzania Ministry of Water report, 2013). The most affected regions include; Arusha, Kilimanjaro, Mara, Manyara, Mwanza, Shinyanga and Singida with Dodoma, Kigoma, Tanga and Tabora are moderately affected by fluoride in water (Malago et
al., 2017). In Arusha, fluoride concentration in surface and groundwater were found to be above 11.0 mg/l in areas surrounding mount Meru including Ngarenanyuki ward (Kloos et al., 1999) whereas, other areas of Tanzania such as eastern and southern regions have fluoride concentrations below 2 mg/l in water sources (Tanzania Ministry of Water report, 2013). Drinking water is often considered to be the main source of fluoride intake by humans and animals (Rango et al., 2012). Furthermore, the regions of northern zones which are Kilimanjaro, Arusha, Tanga and Manyara have been reported to have high prevalence of undiagnosed and untreated Diabetes Mellitus and higher prevalence of community members with glucose impairment who are at increased risk for developing Diabetes Mellitus (Stanifer et al., 2016).

Despite the excessive fluoride consumption has been associated with dental and musculoskeletal defects in humans, none of the studies has been conducted in Tanzania to elucidate any relationship of excessive fluoride ingestion with the emerging Diabetes Mellitus among individuals.

1.2 Statement of the problem

Prevalence of Diabetes Mellitus in Tanzania is on the increase according to demographic survey conducted in Tanzania (Diabetes Association of Tanzania report, 2016). The report shows that changing in life style especially sedentary life (rapid cultural changes), dietary changes, hereditary and decreased physical activity are risk factors that may lead to high incidences of Diabetes Mellitus.

Although most studies were focused on dental and musculoskeletal effects due to excessive consumption of fluoride, several studies have reported fluoride effect on insulin. A study from Lucknow India, showed that excessive fluoride consumption may influence Diabetes Mellitus (Mithal et al., 1993; Pain, 2015). Despite of fact that some areas in Tanzania including Ngarenanyuki ward in Arusha have high fluoride content, such an assessment have not been done to establish any influence of excessive fluoride consumption in the current emerging problem of Diabetes Mellitus in the country.

Therefore, this study was undertaken to examine any relationship between fluoride levels in human blood serum and Diabetes Mellitus status of individuals in specific wards of Tanzania. The results of this study will help to design effective intervention measures in addressing Diabetes health problem in the fluoride endemic areas of Tanzania.
1.3 Rationale of the study

Diabetes Mellitus is a serious challenge to the health system. The contribution of excessive fluoride consumption on occurrence of Diabetes Mellitus remains unclear, furthermore, no any study or very small number of studies have been undertaken in fluoride endemic areas of Tanzania to find out the prevalence of Diabetes Mellitus and its association of excessive fluoride consumption. Therefore data from this study will support the improvement of quality of health care among the Diabetic patients, mostly in the prevention, screening, management and control of Diabetes Mellitus. Furthermore, it will inform key stakeholders better way in allocation of resources regarding Diabetes Mellitus health issues.

1.4 Objectives

1.4.1 General objective

Determine the prevalence of Diabetes Mellitus and assessment of its association with excessive fluoride intake in humans in fluoride endemic areas of Tanzania for future management.

1.4.2 Specific objectives

(i) To assess the Diabetes Mellitus status of individuals in endemic and none endemic fluoride areas.

(ii) Assessment of fluoride levels in blood serum of individuals in endemic and none endemic fluoride areas.

(iii) Impacts of fluoride levels in blood serum on occurrence of Diabetes Mellitus.

1.5 Research questions

(i) What is the magnitude of Diabetes Mellitus status to individuals in endemic and none endemic areas?

(ii) What is the status of fluoride levels in blood serum of individuals in endemic and none endemic fluoride areas?

(iii) What is the impacts of fluoride levels in blood serum on occurrence of Diabetes Mellitus?
1.6 Significance of the study

The results of this research/study, will serve as baseline information/to know contribution of excessive fluoride consumption on occurrence of Diabetes Mellitus and bring awareness to the community, design effective intervention measures in addressing Diabetes problem in Tanzania.

1.7 Delineation of the study

Diabetes health problem is sharply rising in the country and worldwide, most common risk factors for Diabetes Mellitus is a lifestyle, diet, lack of physical activity, hereditary and autoimmune. However, it not much known if other factors including excessive consumption of fluoride can results into reduction of insulin production. There is a limited studies which have investigated the prevalence of Diabetes Mellitus and association of excessive fluoride exposure. The scope of this dissertation is to provide information regarding the prevalence of Diabetes Mellitus and its association to excessive fluoride consumption in Ngarenanyuki fluoride endemic ares – Arusha region population, a cross sectional study with control group which is Mlandizi district (no endemic) – Pwani region.
CHAPTER TWO
LITERATURE REVIEW

2.1 Diabetes mellitus

Diabetes Mellitus is a serious, chronic disease that occurs either when the pancreas does not produce enough insulin (a hormone that regulates blood sugar, or glucose), or when the body cannot effectively use the insulin it produces, is an important public health problem, one of four priority non-communicable diseases (NCDs) targeted for action by world leaders. Both the number of cases and the prevalence of Diabetes have been steadily increasing over the past few decades (WHO, 2012: Care & Supp, 2018).

2.1.1 Diabetes mellitus types

Diabetes Mellitus has two major types. Type one, the pancreas does not produce the amount of insulin essential for survival. It occurs most often in children and adolescents, but can also occur in older people and it is due to distractions of beta cells which produce insulin. Lipid and metabolite profiles can also serve as markers for impending type one Diabetes (Atkinson et al., 2014). These markers include decreased phosphatidylcholine at birth, reduced triglycerides and antioxidant eicher phospholipids followed by increased proinflammatory lysophosphatidylcholine. Higher concentrations of polyunsaturated fatty acid-containing phospholipids, and lower concentrations of methionine in those who developed type one diabetes, are associated with autoantibodies Type two Diabetes is caused by inability of the body to respond adequately to insulin produced by the pancreas. It occurs more frequently in adults, also it has been seen increasingly in children and adolescents (Dey et al., 2016).

2.1.2 Diabetes mellitus signs, symptoms

Signs and symptoms of type one Diabetes Mellitus is usually subtle or no symptoms in early stages, but obvious symptoms are; increased thirst, increased urination, feeling tired, blurred vision and weight loss. Increased thirst, frequent urination, and most severe forms of effects is ketoacidosis or a non-ketotic hyperosmolar state that may develop and lead to stupor and coma (Alberti, 1998), moreover, failure of effective treatment may lead to death.

2.1.3 Medical complications of diabetes mellitus

Diabetes complications it is very important to be known by a Diabetic patient and family members in order to improve the patients’ quality of life (Papadopoulos et al., 2007). The
most common complications are retinopathy, neuropathy, cardiovascular disease, peripheral vascular disease and periodontal disease (King, 2008). Organs and tissues which have abundant capillary vessels are the ones which are severely affected by Diabetes due to the changes that take place in the capillary basement membrane. In type 1 Diabetes the common complications are as a result of microvascular disease while in type 2 Diabetes both microvascular (Kidambi et al., 2008). Most complications associated with Diabetes will occur secondary to the development of microangiopathy (thickening) of the capillary basement membrane (Mcaulay et al., 2001) with associated increased vascular permeability throughout the body. These complications present as diseases of small blood vessels (microangiopathy) or disease of large blood vessels (macrovascular). Microangiopathy and neuropathy causes morbidity and disability through conditions such as blindness due to Diabetic retinopathy, Diabetic foot ulcer (Fig. 1) and bowel and bladder dysfunction and macrovascular complications lead to morbidity through myocardial infarction, stroke, angina, cardiac failure and intermittent claudication (Frier & Fisher, 2006). Almost three quarters of the Diabetic population develop cardiovascular disease and die from cardiovascular complications (Janket et al., 2008). Metabolic control is directly responsible for the severity of Diabetic complications that individual will present with. The duration of Diabetes and other complications may be detected in a patient who is not known to have Diabetes, patient being diagnosed to have Diabetes following a comprehensive investigation testing blood glucose levels (Stan et al., 2010).

### 2.1.4 Diabetic foot ulcer, one of the serious complication

Diabetic Foot Ulcer (DFU) is as a foot (Fig. 1) affected by wound (ulcer) that associated with neuropathy and/or peripheral arterial disease of the lower limb in a patient with Diabetes resulted by three related condition which is neuropathy, ischemia, and infection (Syaril, 2018) with impaired metabolic mechanisms increased the risk of infection and poor wound healing. It happens due to series of mechanisms which include decreased cell and growth factor response, diminished peripheral blood flow and decreased local angiogenesis (Fig. 2). So, the feet are influenced by damage to peripheral nerves, the peripheral vascular disease, ulcerations, deformities and gangrene. Foot problems in Diabetes are common and costly, and people with Diabetes make up about half of all hospital admissions for amputations. In the United Kingdom, people with Diabetes account for more than 40% of hospitalizations for major amputations and 73% of emergency room admissions for minor amputations (American Diabetes Association, 2018).
Figure 1: Diabetic Foot Ulcer image (American Diabetes Association, 2018)

Figure 2: Pathways to Diabetic Foot Ulceration (American Diabetes Association, 2018)
2.1.5 Methods used to diagnose diabetes mellitus

It is still important to understand how the Diabetes Mellitus is diagnosed since there is a significant role to play in identifying those individuals at risk. World Health Organization (WHO) and American Diabetes Association (ADA) has endorsed the screening methods of all those who are at risk for Diabetes (Skamagas et al., 2008). Several tests are used in order to diagnose Diabetes Mellitus but the primary methods are fasting blood sugar levels and oral glucose tolerance tests (Bjelland et al., 2002). Generally, a fasting blood sugar test is used as a way of screening clients, where fasting blood sugar above or equal 7.0 mmol/l is termed as a Diabetic condition (Skamagas et al., 2008).

2.1.6 The global burden of diabetes mellitus

Worldwide, an estimated 422 million adults were living with diabetes in 2014 with global prevalence nearly doubled since 1980, and rising from 4.7% to 8.5% in the adult population (Fig. 3). The prevalence is alarming in low and middle-income countries than in high income countries (WHO, 2016). In Tanzania, Prevalence of Diabetes Mellitus was estimated to be 9.1 %, resulting into unbearable economic burden at family level and the National budget for health care, this burden can be measured through direct medical costs, indirect costs associated with productivity loss, premature mortality and the negative impact of Diabetes Mellitus on nation’s Gross Domestic Product (GDP) (Tanzania Diabetic Association, 2016).
Figure 3: Trends in Prevalence of Diabetes Mellitus, 1980–2014, by country income group
(Global Report on Diabetes WHO, 2016)

Low income communities suffer higher levels of physical ill health more than high income communities and not only is the prevalence and incidence of disease but also the patients follow-up is less successful due to lack of resources (Velupillai et al., 2008). People from low-income groups are vulnerable because they have poor self-esteem, education and emotional poverty which all play a significant role in their state of health. According to Islam et al. (2014), there is a need for interventions that improves knowledge and awareness about Diabetes Mellitus to people who at risk. There are no simple solutions for addressing diabetes but coordinated, multicomponent intervention can make a significant difference (WHO, 2016), everyone can play a role in reducing the impact of all forms of Diabetes. Governments, health-care providers, people with Diabetes, civil society, food producers and manufacturers and suppliers of medicines and technology are all stakeholders. Collectively, they can make a significant contribution to stop the rise in Diabetes and improve the lives of those living with the disease.
2.2 Interactions between diabetes mellitus and fluoride

Fluoride is a naturally occurring mineral found in soil, air and water supply as a result of volcanic activities (Roy et al., 2013). It is capable of reacting with other elements, with multiple implications for human and animal health (Martinez-mier, 2011). Fluoride concentrations widely rich in contaminated water, food and beverages from around the world (Gupta & Ayoob, 2015) and drinking water is often considered to be the main source of fluoride intake by humans and animals (Rango et al., 2012) Studies have shown an evidence of interaction between Diabetes and fluoride whereby endocrine effects due to fluoride exposure, includes decreased thyroid function, impaired glucose tolerance (Diabetes Mellitus), where these effects are achievable with fluoride concentrations in drinking water of above 1.5 mg/l. Impaired glucose tolerance was identified as occurring in humans at fluoride levels 4.9 mg/day (The United States National Research Council report, 2006).

A study done in India, whereby, twenty-five young adults people (age range, 15-30 years) with endemic fluorosis, and an equal number of matched healthy control subjects with normal fluoride intake were studied and 10 of 25 (40%) patients with endemic fluorosis, found with impaired glucose tolerance and significantly higher serum immunoreactive insulin (Trivedi et al., 1993). Another study, in animal model, where sodium fluoride (NaF) in a concentration of 10 mg to 50 mg/l was added to the drinking water of adult Wistar rats and the control group received distilled water. After four weeks, however, in rats receiving fluoride at 50 mg/l glucose uptake in cerebral cortex and hypothalamus was elevated, versus the saline group, fluoride intake had a negligible effect on glucose uptake by peripheral tissues (Rogalska et al., 2017). Another animal study, fluoride exposure affected the oral glucose tolerance test (OGTT) in mice and mechanisms was in glucose homeostasis at the cellular level, in mouse pancreatic cells exposed to fluoride. Mice received 45 mg/l of fluoride through drinking water, and were exposed for 12 hours. Mice showed marginal hyperglycemia an impaired glucose tolerance after four weeks of fluoride exposure, and had significantly lower insulin messenger - ribosomal ribonucleic acid (mRNA) expression, subsequent secretion in the presence of the stimulatory glucose concentration (Garcia et al., 2009) consequently and its secretion from beta-cells which results to hyperglycemia.

2.2.1 Other human health effects due to excessive consumption of fluoride

Human dental and skeletal fluorosis. Crippling skeletal fluorosis is a severe form of skeletal fluorosis and when there is a kyphosis (curvature of the back causing hunchback), scoliosis
(lateral curvature of the spine), genu valgum (one knee is angled in to touch the other knee) and genu varum (outward bowing of the knee), paraplegia (paralysis of lower body), and quadriplegia (paralysis of all four limbs) occurs (Gupta & Ayoob, 2016; Susheela, 2003). Paralysis occurs because of the increased size of the vertebrae bone as well as the narrowing of the spinal canal. The crippling deformities also cause neurological harm and auditory harm, though total deafness is not likely. Decreasing ability to hear is observed because of a compression of the blood vessels affected by scoliosis and narrowing of the auditory canal (Ayoob & Gupta, 2006) but also observed in soft tissues including liver, kidney, and brain (Gm et al., 2008). Individuals with dental fluorosis are those who were exposed to fluoride from birth up to six to seven years of age, exactly the period of tooth formation (Gitte et al., 2015). Animal studies have shown that fluoride crosses the blood brain barrier and accumulates in the brain. Neurotoxicity caused by chronic fluoride exposure in rats results in a marked increase in oxidative stress, and a decrease in the activity of antioxidant enzymes in discrete brain region (Rogalska, 2017).

Fluoride can also lead to non-skeletal fluorosis, such as harmful effects to erythrocytes (red blood cells that transfer oxygen and carbon dioxide between tissues), ligaments, spermatozoa, thyroid glands (regulates rate of metabolism) and destruction of the actin and myosin filaments (motor proteins involved in muscle contractions) in the muscle tissues leading to muscle weakness (Susheela, 2003). The gastrointestinal system is also adversely sensitive to fluoride in drinking water causing gastric irritation such as nausea, vomiting, and gastric pain (Spak, 1989). The gastric pain may be caused by the formation of hydrofluoric acid in the stomach (Susheela, 2003; Ayoob & Gupta, 2006). Fluoride can cause pathological changes such as deoxyribonucleic acid (DNA) damage and lipid peroxidation (Wang et al., 2004). Further, excessive fluoride in water can lead to low hemoglobin levels, excessive thirst, frequent urination, headaches, skin rashes, depression, and negative neurological affects. It affects the brain tissues in a similar way to Alzheimer’s disease (Meenakshi & Maheshwari, 2006). Studies have shown that children that intake excessive fluoride have lower Intelligent Quotient (IQ) scores, impaired cognition and memory, and visuospatial capabilities, especially when it comes to time sensitive tests (Trivedi et al., 2007; Wang et al., 2007; Calderon et al., 2000). Fluoride can affect reproductive system in humans. There have been reported more still and deformed childbirths as well as decrease of birth rates in places with high fluoride concentrations, fluorine can cause changes in the nervous system during pregnancy and can cross the blood brain barrier, accumulating in the brain tissue. This can
cause disruption in synthesis of receptors and neurotransmitters in the cells of the nervous
system (Gupta & Ayoob, 2015). Also, there seems to be a relationship between fluoride and
the morphology and mobility of sperm, levels of testosterone hormones (Ozvath, 2009; Susheela & Jethanandani, 1996).

2.2.2 Importance of fluoride in the human body and recommended rates

Fluoride in limited quantities, is important for general healthy wellbeing, beneficial and
essential to the mineralization of bones and strengthening of dental enamel, increases the
density of bones and also can stimulate the growth of new bone, increases the amount of
minerals in our teeth and even can reverse the progression of dental cavities (Anne & Linda,
2006). The advantages of ingesting fluoride to human health are limited to fluoride levels of
about 1.5 mg/l (WHO, 2004) in potable water, with such levels of fluoride is said to improve
skeletal and dental health, the science behind the beneficial effects of fluoride on the skeletal
structure is based on the ion exchange reactions between hydroxide and fluoride ions in the
calcium hydroxy-phosphate (Malago et al., 2017). The amount of the fluoride ingested each
day, about 90% is absorbed from the alimentary tract and high concentrations of dietary
calcium and other cations form insoluble complexes with fluoride that can reduce absorption
from the gastrointestinal tract (Ramires et al., 2007). About 10% of the daily intake of
fluoride is not absorbed and is excreted in the faeces, except in subjects with high calcium
diets where up to 25% can be excreted by this route (Mullane et al., 2016).

2.2.3 Geographical distribution of fluoride

High concentrations of fluoride in groundwater are found in many countries around the
world, notably (Fig. 4) in Africa, Asia, North America and United State of America,
(Czarnowski, 1996; Azbar, 2000). The most severe problems associated with high fluoride
level in waters occur in China (Wang, 2002) and in Rift Valley countries in Africa (Shupe et
al., 1992). Groundwater with high fluoride contents have been studied in detail in Africa, in
particular Kenya and Tanzania (Moges et al., 1996). Distribution of excessive fluoride in
Africa is high in countries such as Tanzania, Kenya, Algeria, Nigeria, Ghana, Malawi, Sudan,
Uganda, the Republic of South Africa and Ethiopia (Fig. 4).
Figure 4: Areas in the World that have excessive Fluoride in groundwater (Guidelines for Drinking Water Quality, ISBN 92 4 154638 7 - WHO, 2004)

The highest fluoride concentration reported was 2800 mg/l in Lake Nakuru in Kenya (Malago et al., 2017). The occurrence of fluoride in Tanzania was recognized nationally as early as 1950 whereby most of the ground water and surface waters along the rift valley and slopes of the volcanic mountains of Kilimanjaro and Meru were documented (Mbabaye et al., 2016). Most affected regions in Tanzania (Fig. 5) include; Arusha, Kilimanjaro, Mara, Manyara, Mwanza, Shinyanga and Singida and moderately affected Dodoma, Kigoma, Tanga and Tabora (Mjengera & Mkongo, 2003; Malago et al., 2017). In Arusha, fluoride concentration in surface and groundwater were found to be above 11.0 mg/l in areas surrounding mount Meru including Ngarenanyuki ward (Gumbo et al., 1995; Pittalis, 2010) and other areas of the country has low concentration of fluoride in water (Fig. 5) for example, the fluoride concentration in Kibaha District, Pwani region and other southern regions is below 2 mg/l in water sources supply (Tanzania Ministry of Water report, 2013).
However, in Tanzania allowable concentration is 1.5 mg/l with tolerable upper limit at 4 mg/l for human consumption purposes (Tanzania Ministry of Water report, 2013). According to WHO guidelines, 1.5 mg/l is the maximum allowable fluoride water concentration for human consumption purposes (Gordon et al., 2008). Generally, fluoride is found in all natural waters at varying concentrations, however, low and high concentration of fluorides can occur, depending on the nature of the rocks, the occurrence of fluoride bearing rocks and other geological structures (WHO, 2012).
2.2.4 Defluoridation

There is still a great need to upgrade improved sources to safe drinking water all around the world, in both the developed and developing countries. Approximately one-ninth of the people in the world still do not have access to a safe drinking water (WHO, 2004), and population in developing countries lives under acute water scarcity.

There are several current defluoridation methods and can be categorized into four main groups, (a) Coagulation methods, (b) Adsorption methods, (c) Electrochemical methods, and (d) Membrane processes (Gupta & Ayoob, 2016). However, a method that may be sustainable in one community may not be sustainable in other communities because of several factors, such as the stage of urbanization of a community. In industrialized communities, contact precipitation, activated alumina, synthetic resins, reverse osmosis and electrodialysis have been common fluoride removal methods. In developing communities, bone charcoal, contact precipitation, activated alumina and clay have been common effective fluoride removal methods (Fawell et al., 2006). In developing countries, the water treatment systems are mostly decentralized to town, a village plant or household system. Also, there can be a continuous supply of defluoridated water using filters system, like the current nanofilter designed at Nelson Mandela African Institution of Science and Technology (NM-AIST) may serve the purpose at household levels in affected areas and this technology may be efficient, cost effective, environmentally acceptable and easy to use in local communities across Africa (Igor et al., 2019) also this may reduce effects of fluoride in many areas, like Ngarenanyuki ward – Arusha, Tanzania.

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CHAPTER THREE
MATERIALS AND METHODS

3.1 Description of the study area

This study was conducted purposively in Ngarenanyuki ward, Meru district as a fluoride endemic area and Mlandizi ward in Kibaha district as a non-fluoride endemic area (control site).

3.1.1 Ngarenanyuki ward (Fluoride endemic area)

Ngarenanyuki ward is in Meru district, Arusha region and positioned at latitude -3.2151°S, -3.1185°S and longitude 36.8250° E, 36.8841° E. Ngarenanyuki ward comprised of five villages which are Uwiro, Ngabobo, Olkugwado, Kisimiri chini and Kisimiri juu. The ward has a total human population of 20,379 with 10,210 females and 10,169 males (NBS census, 2012). The community uses Engarenanyuki river as the main source of water for human and animal use whereby maximum fluoride concentration was found to be 26 mg/l as reported by Malago et al. (2017) and this water is flowing from the slopes of volcanic Mount Meru. Majority of residents depends on agriculture and livestock keeping as a source of livelihood.

3.1.2 Mlandizi ward (Control site, Non-fluoride endemic area)

Mlandizi ward is found in Kibaha district, Pwani region located at latitude, 6.6986°S, -6.7393°S and longitude 38.6928°E, 38.9929°E. The ward comprised of four villages namely; Yombo, Mbwawa, Mlandizi and Kumba. It has a total human population of 17,318 with 8,772 females and 8,546 males (NBS census, 2012). The ward depends on upper and lower Ruvu water plants as a source of water to the entire population with fluoride concentration below 2 mg/l (Tanzania Ministry of Water, 2010). Mlandizi residents depends on small businesses, agriculture (mainly rice farming) and livestock keeping as sources of their livelihood.

3.2 Study design and sampling

A cross sectional study design was deployed to determine the prevalence of Diabetes Mellitus in relation to fluoride consumption in Ngarenanyuki and Mlandizi wards.

The sample size was estimated using prevalence of 9.1 % (Diabetes Mellitus in Tanzania) as reported by Tanzania National Survey (2018). Therefore, a proportion of 9.1% prevalence was used to calculate the sample size as described by Daniel (1999) cited by Naing et al. (2006).
\[ N = Z^2 P (1 - P) \frac{E^2}{N} \]

Where \( E = \) Marginal error (0.05) \( Z = (1.96) \); Prevalence, \( P = 9.1\% \)

\[ N = 3.84 \times 0.091 (1 - 0.091) \]

\[ 0.0025^2 \]

\( N = 127. \) Therefore, at prevalence of 9.1\% a total number of 127 individuals were required to be involved from each site. Individuals were randomly selected from each population. However, the Inclusion criteria includes, individuals \( \geq 25 \) years old, not seriously sick at the time of visit, voluntarily consent to participate and lived in either Ngarenanyuki or Mlandizi ward for more than 3 years. Also, individuals who were asked to fast for blood glucose test and violated were excluded from the study.

3.3 Field samples collection

3.3.1 Individual sampling

From the sampling structure, 150 individuals from Ngarenanyuki and 150 from Mlandizi wards were randomly selected based on multistage sampling of villages, sub-villages and households. Three villages, two sub-villages from each village and five households from each sub village were selected randomly for the study.

3.3.2 Blood sampling and fasting glucose test procedure

One day prior sampling households (Fig. 6), were visited and individuals who agreed to participate in the study were asked to fast overnight (at least 8 hours) until morning when blood samples were collected. Briefly, blood samples were collected in the morning hours between 0600 and 1000 hrs (Fig. 7). Participant venous blood of approximately 4.5 mls were collected from the brachial vein and kept in a plain test tube which allows serum separation for fluoride determination. Then a drop of capillary blood sample was taken and put an ON CALL PLUS Automatic glucometer machine, distributed by CLIA waived TM, 11 578 Sorrento Valley Road, suite 25/26 San Diego, CA 92121 for fasting glucose testing determination. The results of fasting blood sugar test were displayed in mmol/l and documented well in excel sheet.
Figure 6: Household sampling and preparation of individuals for the Fast Blood Sugar (FBS) test at Ngarenanyuki ward, Meru District.
3.4 Determination of fluoride levels in human blood sera

Determination of inorganic fluoride in human sera took place at NM-AIST laboratories using an Ion-Selective Electrode (ISE) as described by Klesa (1987).

3.4.1 Preparation of buffer

Total Ionic Strength Adjustment Buffer (TISAB II) was used at the ratio of 1:1. TISAB II was briefly prepared by taking 500 ml of distilled water in a 1 litre beaker. Then 57 ml of glacial acetic acid and 58 g of Sodium chloride were added. Also, 4 g of Cyclohexanediylbis [N-(carboxymethyl), monohydrate, Trans-1, 2-cyclohexanediamine-tetraacetic acid monohydrate (CDTA)] was added and stirred to dissolve. The mixture was placed in a cool water bath whereby, 125 mls of 6N NaOH was added while stirring slowly until pH was between 5 – 5.5. Calibration was done by using standard stock solution with the dilution of concentration ranging from 0.01 up to 1 mg/l.
3.4.2 Determination of fluoride levels in human sera

The sera were obtained after centrifuging blood samples for 10 min. at 3000 rpm. Serum from each tube was decanted into Eppendorf tubes and then stored in a freezer at -20ºc awaiting analysis. Briefly, the Total Ionic Strength Adjustment Buffer (TISAB II) was mixed with sera at the ratio of 1:1 and measured while under magnetic stirrer by using a minitype fluoride ion selective Electrode (ISE) manufactured by Digisystem Laboratory Instrument, Inc. Taiwan (Fig. 8). Instruments and apparatus were rinsed thoroughly with distilled water to ensure no traces of blood serum left outside before another consecutive measurement was done. Results were displayed in mg/l and documented in the excel sheet.

Figure 8: Determination of fluoride in human sera using Ion-Selective Electrode (ISE) at NM-AIST Laboratories
3.5 Ethical research clearance

Prior to the research work, an ethical clearance with certificate number NIMR/HQ/R.8a/Vol. IX/2854 was obtained from the National Institute for Medical Research (NIMR), Dar Es Salaam-Tanzania. Community sampling permission was granted by the District Executive Director (DED).

3.6 Data analysis

The data were handled by using Statistical Package for Social Sciences (SPSS) version 20 and then transferred into STATA version 13 for processing and analysis. Categorical and Numerical variables were summarized by using frequencies, proportions and measures of central tendency. Student’s t-test was employed to compare means of Fasting Blood Sugar levels and Fluoride levels in blood serum. Chi – Square test and Karl’s Pear correlation were used to establish relationship between Fasting Blood Sugar levels and Fluoride levels in blood serum.
CHAPTER FOUR
RESULTS AND DISCUSSION

4.1 Study results

4.1.1 Fasting blood sugar test results

Reference values described by World Health Organization (2006), the capillary Fasting Blood Sugar (FBS) of ≥7.0 mmol/l (126 mg/dl) is considered as having Diabetic condition. A total of 300 study participants, were enrolled and screened for Fasting Blood Sugar (FBS) from both study population (Ngarenanyuki fluoride endemic and Mlandizi ward non-endemic). 25 (8.5 %) had FBS ≥ 7 mmol/l, these were considered to be in Diabetic condition. 64.0% of individuals with FBS ≥ 7 mmol/l, were residing in Mlandizi (Fig. 9) Based on gender, participants screened for Diabetes (FBS) from both two sides, high proportions were females, 64.0% (Fig. 10) Mlandizi and 51.3 % Ngarenanyuki

![Figure 9: Fasting blood sugar by residence (n=300)](image)
The mean age of participants screened for Diabetes (FBS) from both sides Ngarenanyuki and Mlandizi ward was 51.7 ± 14.0. High proportion who had FBS ≥7.0 mmol/l was people above 50 yrs of age (Fig. 11).

In Ngarenanyuki, fluoride endemic area, total of 141 individuals had fasting blood sugar (FBS) < 7 mmol/l the proportion of 94.0% (Fig. 12)
The fluoride levels in human blood serum were described with the values of 0.06 mg/l as normal reference values (WHO, 2004; Kanduti et al., 2016). Total of 97 participants from both two sides consented for this test. People who had fluoride level in blood serum > 0.06 mg/l is 29 (30%) and 79.30% of them was from Ngarenanyuki ward (Fig. 13), female led by having proportion of 51.7% with significant difference (p < 0.049). In Mlandizi ward, fluoride non-endemic area (n = 47), total of 6 individuals had fluoride levels in blood serum > 0.06 mg/l, the proportion of 12.80% (Fig. 15). Fluoride levels in blood serum ranged from 0.02 to 0.22 mg/l, with Ngarenanyuki having the median 0.054 mg/l and Mlandizi 0.041 mg/l.

Figure 13: Proportion of fluoride levels in both study Ngarenayuki and Mlandizi (n=97)
In Ngarenanyuki, those with fluoride levels in blood serum \( \leq 0.06 \text{ mg/l} \) proportion of 70.4% were aged > 50 years and among those with fluoride levels in blood serum > 0.06 mg proportion of 43.5% were also aged > 50 years (Fig. 14).

Figure 14: Proportion of Fluoride levels in blood serum by age in Ngarenanyuki ward (n=50)

Figure 15: Proportion of fluoride levels in blood serum in Mlandizi (n=47)
(i) Relationship between Fasting Blood Sugar level and fluoride levels in blood serum  
Based on the Chi Square Test - with Cross Tabulation analysis

Based on the Chi Square Test - with Cross Tabulation analysis (Table 1) the relationship between fasting blood sugar level and fluoride levels in blood serum at Ngarenanyuki ward and Mlandizi (P < 0.747)

Table 1: Chi Square Test - with Cross Tabulation α = 0.05

<table>
<thead>
<tr>
<th>Human Serum Fluoride</th>
<th>Fasting Blood Sugar</th>
<th>Total</th>
<th>P - value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Fluoride levels in blood serum (Ngarenanyuki &amp; Mlandizi) (n=97)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Normal (≤ 0.06 mg/l)</td>
<td>60 (70.6)</td>
<td>8 (66.7)</td>
<td>68 (70.1)</td>
</tr>
<tr>
<td>Abnormal (&gt; 0.06 mg/l)</td>
<td>25 (29.4)</td>
<td>4 (33.3)</td>
<td>29 (29.9)</td>
</tr>
<tr>
<td>Fluoride levels in blood serum (Mlandizi) (n=47)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Normal (≤ 0.06 mg/l)</td>
<td>36 (87.8)</td>
<td>5 (83.3)</td>
<td>41 (87.2)</td>
</tr>
<tr>
<td>Abnormal (&gt; 0.06 mg/l)</td>
<td>5 (12.2)</td>
<td>5 (16.7)</td>
<td>6 (12.7)</td>
</tr>
<tr>
<td>Fluoride levels in blood serum (Ngarenanyuki) (n=50)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Normal (≤ 0.06 mg/l)</td>
<td>24 (54.5)</td>
<td>3 (50.0)</td>
<td>27 (54.0)</td>
</tr>
<tr>
<td>Abnormal (&gt; 0.06 mg/l)</td>
<td>20 (45.5)</td>
<td>3 (50.0)</td>
<td>23 (46.0)</td>
</tr>
</tbody>
</table>

(ii) Relationship between Fasting Blood Sugar level and fluoride levels in blood serum  
Based Pearson’s correlation coefficient analysis

Based on the Pearson’s correlation coefficient analysis association between fasting blood sugar level and fluoride levels in blood serum at Ngarenanyuki ward (n = 50) (r = - 0.0632; P < 0.663) (Fig. 16).
4.2 Discussion

4.2.1 Diabetes mellitus status of individuals in endemic and none endemic fluoride areas

The prevalence of Diabetes Mellitus reported in this current study was 8.3%, less than that of Tanzania National Diabetes Mellitus prevalence of 9.1%, the reason might be associated with the fact that this screening of Diabetes in the present study was based on community epidemiological screening (fieldwork), focused on single test (FBS), where National Diabetes Mellitus prevalence records are generated from hospitals. Prevalence of 8.3%, does not mean Diabetes health problem has decreased, the current study has similar trend, agreed with study conducted in Tanzania Northern Zone of the huge burden of undiagnosed, untreated Diabetes Mellitus and higher prevalence of community members with glucose impairment who are at increased risk for developing Diabetes Mellitus (Stanifer et al., 2016) where Non-communicable diseases like Diabetes is growing very rapidly and many healthcare systems are struggling to deal with communicable diseases such as HIV and Malaria.

Diabetes Mellitus to affect aged people is very common. In this current study, high proportion (64%) of (FBS) ≥7.0 mmol/l individuals found in Mlandizi ward, and based on age group, people above 50 yrs of age screened to be in Diabetic condition, this line with another studies where, it was reported that the reason may be because of combined effects of
increased insulin resistance and impaired pancreatic functions in people as they grow old (Zhao et al., 2013). Moreover, in actual practice in Africa this elder group, apart from being vulnerable due to old age, many old people fail to access medical attention, this may be due to poor health systems and lack of awareness in the general population. A study conducted in Kenya, on health seeking behavior, it was found out that negative attitudes of healthcare workers were associated with elder people delaying seeking healthcare (Waweru et al., 2003). Another barrier is cost implication, if elderly patients with Diabetes Mellitus are not exempted from payments, would be impossible for most elderly patients to meet the cost of insulin and oral hypoglycaemic drugs.

4.2.2 Fluoride levels in blood serum of individuals in endemic and none endemic fluoride areas

From the present study population, individuals with fluoride level in serum above 0.06 mg/l were 52% prevalence, 79.30% came from Ngarenanyuki ward compared to Mlandizi populations of 20.70%. These results show widely problem of environmental fluoride contamination, here talking about high fluoride in human body, this line with another study done at the same place, Ngarenanyuki (Mkungu et al., 2014) revealed, analysis of fluoride in soil composition were 4 mg/l to 10 mg/l for the Ngarenanyuki ward and Ongadongishu ward respectively. This reflect that fluoride implication in the area is serious because people depends on horticulture of which soil has been used for, it is fully of fluoride contamination eventually human health defect. In Ngarenanyuki ward, fluoride levels in serum, there was gender significant difference (p < 0.049) with female had higher fluoride levels in serum. This line with another study conducted at Ngarenayuki whereby, Women aged between 15-45 years old were involved, identified water used for domestic purposes was the leading (68.63%) route of fluoride contaminant (Joseph et al., 2019), and the majority (70.6%) of the participants were not aware of the potential sources of fluoride contaminants such as grown food crops/vegetables this end up by women consumed fluoride excessively because do not have knowledge about fluoride contamination. Furthermore, the study revealed that women participants were at high level of fluoride exposure than men this could be possibly through fluoridated contaminated domestic water, grown food crops/vegetables, possibly, it is due to African culture, that all surveyed villages women occupies mostly in housekeeping and domestic family wellbeing. Also the findings are similar with another study whereby, gender
related difference of fluoride levels in serum were found in two different fluoride endemic villages but not with age related difference (Xiang et al., 2004).

The current study also revealed the decrease of fluoride in serum with age, line with study which showed children with age below 10 years suffer much with health defect consequences mostly dental and skeletal fluorosis due to excess fluoride consumption but health impacts is less at older age (Yoder et al., 1998). However, in this study, age of participants involved was ≥25 years and hence children could not be observed.

4.2.3 Impacts of fluoride levels in blood serum on occurrence of diabetes mellitus

In this present study, relationship could not be elucidated between fluoride on occurrence of Diabetes Mellitus based on the (Pearson’s correlation coefficient \( r = -0.0632; P < 0.663 \)) analysis between fasting blood sugar levels and fluoride levels in serum of the study population, which indicated that there was no significant correlation. This result is different from another study done in lucknow, India whereby twenty-five young adults (age range, 15-30 years) with endemic fluorosis, and an equal number of matched healthy control subjects with normal fluoride intake were studied and impaired glucose tolerance was demonstrated in 10 of 25 (40%) patients with endemic fluorosis (Trivedi et al., 1993 with the results of endemic fluorosis patients to have significantly higher glucose levels of 7.8 to 11.0 mmol in serum (on the 75-g oral glucose tolerance test - OGGT)). Oral glucose tolerance test is test whereby a healthcare provider takes a fasting blood of an individual and to test for glucose level first, then ask patient to drink a cup (8 ounces) of a syrupy glucose solution that contains 75 grams of sugar, then after two hours to repeat test where normal Fasting Blood Sugar (FBG) should be less than 7.8 mmol/l (WHO, 2006). Also in animal model, study on mice, a reduction in insulin messenger-ribosomal ribonucleic acid (mRNA) and its secretion from beta-cells which results to hyperglycemia (Garcia et al., 2009) mechanisms was in glucose homeostasis at the cellular level, in mouse pancreatic cells exposed to fluoride. Mice received 45 mg/l of fluoride through drinking water and were exposed for 12 hours. Mice showed marginal hyperglycemia an impaired glucose tolerance after 4 weeks of fluoride exposure, had significantly lower insulin messenger - ribosomal ribonucleic acid (mRNA) expression and subsequent secretion in the presence of the stimulatory glucose concentration. At Ngarenanyuki ward, where water contains fluoride more than 1.5 mg/l (Malago et al., 2017). Lack of association may be attributed to milk consumption as most households keep cattle and small ruminants for livelihoods, therefore, for their daily diet, consumption of cow
milk it is normal habit of Ngarenanyuki people. Calcium in cow milk has been reported to form insoluble complexes with fluoride that can reduce absorption from the gastrointestinal tract (Ramires et al., 2007). About 10% of the daily intake of fluoride is not absorbed and is excreted in the faeces, except in subjects with high calcium diets where up to 25% can be excreted by this route (Mehta, 2013).

Similar results have been found in a study done in Turkey, the results indicated that there was no danger of fluorosis developing from the consumption of milk produced in the Çaldıran and Doğubeyazıt districts where fluoride levels were significantly higher (p < 0.05) in spring (Ocak & Kösea, 2018), however, the consumption of drinking water from these districts, with its excess of fluoride causes fluorosis, including dental fluorosis, skeletal fluorosis, and non-skeletal fluorosis. It is necessary that measures are taken to reduce this risk and provide safe drinking water in Ngarenanyuki population.

In addition, taking into other consideration of Ngarenanyuki ward, lack of association could be also due to sample size focused in study area during community epidemiological screening, and there, may have confounding factors that could be uncovered.
CHAPTER FIVE

CONCLUSION AND RECOMMENDATIONS

5.1 Conclusion

Results of the present study revealed that fluoride in the blood serum of individuals at Ngarenanyuki ward it is in high levels, although fluoride did not show significance influence on Diabetes Mellitus, it is well known fluoride has a serious different health implications, especially for the women and children. This current study shows, Women are much exposed in endemic area than men, it is simply because in Africa, women are engaged much in production activities, and they are backbone of their families, similarly to the country’s economy at large. Immediate measures are recommended to minimize the threat of fluoride at the household level. Community should be encouraged to use non-fluoridated water to grow vegetables and (domestic) household consumption. Strategic approaches should be deployed to alleviate the current situation for removing excessive fluoride from water, such approaches may include different technologies available, for example, use of the current nanofilter designed by NM-AIST may serve the purpose. Prevalence of Diabetes Mellitus reported in this current study was 8.3%, it is close to prevalence of Tanzania National Diabetes Mellitus prevalence of 9.1%, this does not mean Diabetes health problem has decreased, but this shows similar trend of unsurveyed, undiagnosed of Diabetes Mellitus burden in Tanzania. Therefore, it is advocated to raise awareness and training across the population on how to fight Diabetes Mellitus health problem.

5.2 Recommendations

Findings of this current study has shown fluoride in blood serum of individuals in Ngarenanyuki ward it is in high levels with a different health implications. Therefore, it is recommended that:

(i) Ngarenanyuki community should be encouraged to use non-fluoridated water for (Domestic) household consumption.

(ii) And Government should put strategies to alleviate the current situation for removing excessive fluoride from water.

(iii) Health education on prevention of Diabetes Mellitus health problem should be advocated to entire community.
(iv) Fluoride endemic community should be encouraged to continue with livestock keeping industry as it has been noted that cow milk in diet promote human health also helps in fluoride absorption as a result decreases fluoride toxicity.
REFERENCES


