بسم الله الرحمن الرحيم

Sudan Academy of Sciences (SAS)
Atomic Energy Council

Studies on Simple Goitre Epidemiology and Aetiology in Sudan

Presented By

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M. Sc. Physiology
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1993

A Thesis Submitted to the Sudan Academy of Sciences in Fulfillment of the Requirements for the degree of Doctorate in Physiology

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2008

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Date of Examination: June 2008

Dedication

To my parent's soul, who spent their lives to provide us with the shelter, dignity and the light to find our way. To my uncle Osman Geneid, Rabee, my brothers, sisters, friends. To my sons and my wife. I dedicate this work with endless love.

Acknowledgements

I would like to express my deep gratitude to professor Amal Mahmoud Saeed for her invaluable knowledge, support, patience and encouragement throughout the thesis. I am also very grateful to Dr Abdel Salam Elnour for his endless support and guidance. My thanks are also extended to Dr Fathi A/Raouf Elkhangi for his generous advice and continuous support. I am grateful to the school principals and pupils who participated in the study. I would also like to thank the Directors, Education and health authorities in the selected cities for their assistance in the administrative part of the research. My thanks are also extended to all my colleagues in Sudan Atomic Energy commission for making this work possible among those, Tayseer Yagoub, Sulafa Kamil, Manal Mahgoub, Alawia Ballah, Nuha M Elhassan, Anwar, Hala Madani and Nahid Shabo, Hamid S. Hamid, Nagi I. Ali, Mohammed Elimam and Elfatih Yagoub.

Special thanks for Professor Mohammed Ahmed Hassan Eltayeb the director general of SAEC and Dr. Eltayeb Ahmed Eltayeb the director of Radiobiology Institute for the generous grant and giving me the time to do this study. I am much grateful to Natalina Wadelreef, Osman Mahgoub, Faisal Omer, Abbas Elatta, Waleed A/Bagi, A/Raouf Morad, Elwaleed Yousif and Mohammed Gamal for their contribution and commitment in the fieldwork. I am also grateful to Dr. Ammar Khamees, Dr. Isam Salih, Zaki A/Kareem and Mohammed A/Alla for their skilled statistical analysis.

I am much indebted to my colleagues Ammar M. Alamin and Manal Hasab Elrasoul for giving me their time for computer assistance throughout the study.

I would like to thank also the Laboratories Department of the Groundwater & Wadis Directorate for the analysis of water samples.

Abstract

The objectives of this study were to map the prevalence of goiter in Sudan and to study the etiological factors involved. A further objective was to explore the use of serum thyroglobulin (Tg) level in the assessment of endemic goitre.

The survey took place in the period from June to November 2006. The survey covered nine cities including Nyala and Elfasher (Western part), Wau (in The South), Atbara and Dongula (in the North), Dmazine (South East), Port Sudan (Eastern part), Kosti and Khartoum (in the Centre). Khartoum was divided into three different cities Khartoum, Khartoum North and Omdurman. The study included 6181 male and female schoolchildren at the age of 6-12 years old. All the children were clinically examined for the presence of goitre using WHO palpation method.

Blood samples were randomly collected from 360 children (30-37) from each selected city irrespective of their thyroid status or gender. Serum samples were analyzed for the concentration of T_4, T_3, TSH, and Tg.

Casual urine samples were also collected from the same selected subjects. Urine samples were analyzed for iodine and thiocyanate concentrations.

Water samples were collected from each school and analyzed for the concentration of Ca, Mg, Cl, F and total Hardness.

The results indicated that the overall total goitre rate was 40.62%. The highest goitre rate was found in Kosti town (77.67 %) and the lowest in Omdurman "Khartoum state" (12.22%). The overall median urinary iodine excretion (UIE) was 6.55 µg/dl. Iodine deficiency was detected in 70.28% of the children and there were great variations in the median UIE from region to another started from as low as 2.70 µg/dl in Kosti to 46.40 µg/dl in Port Sudan city (at the coast of the Red Sea). The overall median concentration of urinary thiocyanate was 0.37 mg/dl. There were also variations in the median levels of urinary thiocyanate from city to another

and slightly exceeded the cut off point (0.46 mg/dl) in pupils from Wau and Nyala cities.

The Mean±SD serum concentration of thyroid related hormones T_4, T_3, TSH and Tg were 103.23±28.15 nmole/l, 1.94±0.52 nmole/l, 2.25±1.58 mu/l and 38.0±22.1 ng/ml respectively. They were all within the normal reference range.

Water samples analyses indicated that, Calcium, Magnesium, total Hardness, Chloride and Fluoride ions were present in high concentrations that exceeded in some samples the maximum permissible concentrations. There were strong correlations between water chemical components and the thyroid related hormones as well as with the goitre prevalence. The results of this study indicated that the high concentrations of water chemicals mainly Ca and Cl are strongly interfering with the iodine metabolism and consequent thyroid function and anatomy. There were no statistical correlations between goitre prevalence and urinary iodine or thiocyanate execrations. The results of thyroid related hormones indicated that serum T_4, T_3 and TSH were in the normal range in iodine deficient pupils. Measurement of these hormones did not reflect the iodine status or goiter prevalence in the populations studied. Thus, determination of these hormones was not the suitable indicator for goitre monitoring programs. Although there was no statistical correlation between serum thyroglobulin concentration and the prevalence of goitre, the highest serum thyroglobulin concentrations were found in Kosti where the iodine intake was minimum, and in Port Sudan where high concentrations of water chemicals overt antithyroid activity. The serum thyroglobulin level in this study as well as the urinary iodine concentration can be taken as base-line data for the iodine supplementation program.

الخلاصة

من الأهداف الرئيسية لهذه الدراسة معرفة المعدل الكلى لإنتشار تضخم الغدة الدرقية (الدراق) الراهن فى السودان والأسباب المؤدية للمرض. ومن الأهداف الأخرى معرفة الوضع التغذوى لليود من خلال قياس مقدار اليود فى البول. كما تهدف الدراسة أيضا الى قياس بروتين الثيروقلوبيولين فى عينات الدم ومعرفة إمكانية إستخدامه كمؤشر حيوى لمعرفة اثار عوز تناول اليود.

غطت الدراسة عدة مدن سودانية شملت كل من واو ، الفاشر ، نيالا ، الدمازين ، كوستى ، عطبرة ، دنقلا ، بورتسودان والخرطوم فى الفترة من يونيو إلى نوفمبر من العام 2006م .

شملت الدراسة 6181 تلميذ عمر 6 إلى 12 عام من الجنسين . أخضع كل الأطفال للفحص الطبى لمعرفة وجود مرض الدراق أو عدمه .

تم جمع عدد من عينات الدم عشوائياً من عدد من التلاميذ من كل مدرسة وكذلك عينات بول لنفس الأشخاص الذين تم جمع عينات الدم منهم . تم أيضاً جمع عينة مياه شرب من كل مدرسة لمرفة مستوى العناصر الكيميائية التى شملت عناصر الكلور، الكالسيوم، الماغنزيوم، الفلور وكذلك عسر الماء .

تم قياس هرمونات الغدة الدرقية الثايروكسين و الثايرونين ثلاثى اليود وكذلك الهرمون المحفز للغدة الدرقية وبروتين الثيروقلوبيولين فى عينات الدم . كما تم أيضاً قياس مستوى اليود وكبريتات السيانيد فى البول .

من النتائج التى توصلت لها هذه الدراسة أن النسبة الكلية لإنتشار الدراق فى المدن المختارة هى 40.62% كما أن أعلى المعدلات كانت فى مدينة كوستى إذ وصلت إلى 77.67% وكانت أقلها فى مدينة أمدرمان 12.22% . وكانت القيمة الوسطية لمقدار اليود فى البول هى 6.55 مكروغرام فى كل 100 مللتر مع وجود إختلاف كبير لهذه القيمة من مدينة الى أخرى حيث كانت أقل القيم فى كوستى (2.7 مكرغرام) وأعلاها 46.4 مكروغرام فى كل 100 مللتر فى بورتسودان .

أثبتت الدراسة أن 70.28% من الأطفال يعانون من عوز تناول اليود .

وجدت الدراسة أن القيمة الوسطية لمقدار كبريتات سيايند فى البول هى 0.37 ملغرام فى كل 100 مللتر ، كما أن أقل القيم فى بوتسودان (0.26% ملغرام) وأعلاها فى واوحيث وصلت الى 0.46ملغرام فى كل 100 مللتر.

أما تحليل الهرمونات فى عينات الدم نتج عنه أن متوسط هرمون الثيروكسين هو 112.78 ± 22.83 نانو مول فى اللتر و 1.90 ± 0.40 نانو مول فى اللتر للثيارونين ثلاثى اليود ، 1.83 ±

1.04 وحدة فى اللتر للهرمون المحفز للغدة الدرقية و 23.3 ± 11.2 ملغرام لكل مللتر لبروتين الثايروقلوبيولين .

وجدت الدراسة أن هرمون الثايروقلوبيولين مرتفع فى 71.94% من الطلاب مما يتناسب ونسبة عوز اليود عند التلاميذ .

وجدت الدراسة أيضاً أن العناصر الكيميائية الموجودة فى عينات مياه الشرب مرتفعة فى بعض المناطق مما كان له الأثر فى زيادة إنتشار معدل الدراق حتى فى بعض المناطق التى يتواجد فيها عنصر اليود بكميات وفيرة مثل بورتسودان.

أثبتت الدراسة عدم وجود علاقة ملموسة بين تركيز هرمونات الغدة الدرقية فى الدم و نسبة إنتشار الدراق.

كما أن تركيز هذه الهرمونات لم يتعدى المستوى الطبيعى حتى عند الأطفال الذين يعانون من عوز اليود الحاد، عليه تقترح الدراسة عدم جدوى قياس هذه الهرمونات فى معرفة التأثيرات السالبة على الغدة الدرقية أو إستعمالها كدلالات فى مسح إنتشار الدراق .

أثبتت الدراسة أن إنتشار الدراق يعتمد على عدة أسباب منها البيئية مثل ذيادة تركيز بعض العناصر الكيميائية فى مياه الشرب والتى تعمل على تثبيط قدرة الغدة الدرقية فى إمتصاص مادة اليود فى مناطق وإنتشار تناول الدخن والذرة مما ينتج عنه زيادة إستهلاك كبريتات السيانيد فى مناطق أخرى إضافة لعوز تناول اليود والذى يعتبر السبب الرئيسى فى إنتشار الدراق .

إستنتجت الدراسة أن مستوى هرمون الثايروقلوبيولين كان مرتفعاً عن المعدل الطبيعى عند أكثر من 70% من التلاميذ ، وعليه يمكن أن نعتبره قاعدة بيانية أولية تبنى عليها الدراسات المتوقعة القادمة و المتعلقة باذالة عوز اليود.

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ABBREVIATIONS

ICCIDD: International Council for Control of Iodine Deficiency Disorders

IDD: Iodine deficiency disorders

RDA: Recommended dietary allowance

T_1: Monoiodotyrosin

T_2: Diiodotyrosin

T_3: Triiodothyronine

T_4: Thyroxin

Tg: Thyroglobulin

TGR: Total goitre rate

TPO: Thyroid peroxidase

TRH: Thyrotropin-releasing-hormone

TSH: Thyrotropin, Thyroid-stimulating hormone

Tvol: Thyroid volume

UIE: Urinary iodine excretion

USCN: Urinary thiocyanate

UNICEF: United Nations Children's Fund

USI: Universal salt iodization

WHO: World Health Organization

CHAPTER ONE

INTRODUCTION

1.1 Problem statements

Iodine deficiency in Sudan

The endemicity of goitre in Sudan was firstly reported by Woodman in 1952 (Woodman, 1952). He reported the presence of endemic goitre in a small area inhabited by the Zandi group in southern Sudan near the border with Congo Democratic Republic (Zaire). He also reported high goitre rate among the Newir tribe in the south of Malakal. Woodman also reported goitre endemicity around Eldamer in the northern province and in Darfur in the western Sudan. In 1967 Kambal conducted an extensive survey which included 17470 people in Darfur province (Kambal, 1969). Kambal reported that 57% were goitrous and 18.5% of these had large goitre. He also reported in the same study that 12.6% of 5566 subjects were found to have goitre in Khartoum. The incidence of endemic goitre among schoolchildren in Darfur was 85 % (Eltom, 1984). Eltom also reported that the endemicity of goitre started to spread throughout the country. He also noted a new focus of endemic goitre in Kosti area in central Sudan (Eltom *et al.*, 1985).

In 1997 a comprehensive national survey performed by The National Nutrition Directorate under the Federal Ministry of Health (the focal point and responsible for the IDD control program in Sudan) in about 41,000 primary schoolchildren (FMOH, 1997). The overall total prevalence of goitre in Sudan reported in that survey was 22%. The detail of goitre rate for each region reported in the survey was 38% in the Northern region, 8% in Eastern region, 5% in Khartoum, 23% in the Center, 28% in Darfur, 39% in Kordofan, with the higher prevalence of 42% in the Upper Nile region. Bani (2006) in his report referred to the surveys performed by Eltom and others in the 1980s that total goitre prevalence was ranging from 87% in Darfur State and 78% in Central Region to 17% and 13% in Khartoum and Port Sudan respectively. He also reported that, each year 242,400 Sudanese children are born without protection from the consequences of iodine deficiency.

Among this affected group, IDD may lead to cretinism in 3 per cent (7000), severe mental impairment in 10 per cent (24,000) and mild intellectual incapacity in 87 per cent (210,000) (Bani, 2006).

In an epidemiological study (Elnour *et al.*, 2000) in preschool children in the southern Blue Nile area of Sudan. Goitre was endemic in that region of the country despite iodine sufficiency. The goitre rate was 22.3%. The median urinary iodine concentration was 0.79 mmol/L and 19.3% of the children had a concentration > 1.57 mmol/L. The study revealed that anemia and vitamin A deficiency are health problems in the area. Moreover, consumption of millet, vitamin A deficiency, and proteinenergy malnutrition are possible goitre etiologic factors in that goitre endemic area. It was reported that 30 million Sudanese out of 34 are at risk of IDD. More than 1 million (99%) newborns not protected against brain damage every year. About one quarter of these born with IDD consequences. They are suffering from reduced learning capacities, poor school performance and increased drop out rates (Bani 2006).

Control of IDD in Sudan

IDD control programs in Sudan started in the mid 1970's with distribution of iodine capsules as a main target. The majority if not all of these programs are no longer existing now. The period from early 1980th to the late 1990th had witnessed a number of goitre surveys including goitre etiology and distribution of iodine in different forms in large parts of Sudan (Eltom, 1984; Abdel Wahab *et al.*, 1984; Abdel Monim 1993; Elnagar *et al.*, 1995; Elnour et al., 2000). The Western part of the country (specially Darfur region) was extensively studied. Southern, Northern and Eastern parts of the country were poorly referred to in the previous studies.

In 1989, a National IDD Program was launched. The Program involved nationwide surveys of iodine deficiency and finding of possible solutions to the problem. Further steps by the government led to the setting up of an inter-agency

High Technical Committee, which was given the task of drawing different strategies for the elimination of, or at least control the levels of iodine deficiency by introducing different iodine supplementation techniques including fortification of salt, sugar and water with iodine (Bani, 2006).

A technical committee for IDD control was set up in 1991 and given the tasks of distributing iodized oil as well as establishing a long-term strategy for iodizing salt. In 1992 the High Technical Committee approved the salt iodization as a long-term sustainable strategy (Bailey *et al.*, 1992; Eltom *et al.*, 1994) and to support the ongoing studies in iodination of water and sugar. In 1994 Sudan adopted Universal Salt Iodization (USI) as a National IDD prevention strategy, the main objective of the program was to fortify all edible salt with iodine to a level of 50ppm using potassium iodate (KIO_3). Several amendments have been issued since but not enforced. On the seventh of July 2003 a Ministerial Declaration (13/2003) was issued under the 1975 Public Health Law, provides for the blending of salt with Potassium Iodate (at 30 mg/kg). All salt producers were called to ensure that all salt produced in Sudan will be iodized within six months of the date of the declaration. On the third of January 2004 salt was allowed to be iodized with either Potassium Iodate or Potassium Iodide. Different iodization equipment and materials were provided by international development organizations such as United Nations Children's Fund (UNICEF) to support the USI program (Eltom *et al.*, 1994). Bani (2006) revealed in his report that, these resources have not been optimally applied and there was no any progress has been made in the implementation of the strategy since. It has been estimated by the UNICEF that currently only 1% of households have access to adequately iodized salt (Hussien, 2006).

1.2 OBJECTIVES OF THE STUDY

General objectives

1. To map the prevalence of goiter in the Sudan and to explore the etiological factors involved in different parts of the country.
2. To evaluate serum thyroglobulin (Tg) levels in endemic goiter areas.

Specific objectives

1. To determine the prevalence of goiter in school children in nine selected cities of Sudan
2. To evaluate the contribution of iodine deficiency, dietary goitrogens and drinking water constituents to the etiology of endemic goiter
3. To determine some of the factors that affect serum thyroglobulin in endemic goiter areas

1.3 Research methodology

In this study we will follow the criteria of goitre survey recommended by the WHO (WHO 1994) that thyroid enlargement will be detected in large populations of schoolchildren in the age range of 6 to 12 years old in a number of selected Sudanese cities representing different geographical and socioeconomical back grounds.

Blood and urine samples will be collected and analyzed for some analytes to measure the thyroid gland status and to detect the effects of some environmental factors on the thyroid functions.

Water samples will be collected from the participated schools to measure the concentrations of some chemicals and to detect their possible roles in the aetiology of goitre.

CHAPTER TWO

LITERATURE REVIEW

2.1 Iodine

Iodine is an essential trace element widely distributed in nature, and essential to the formation of thyroid hormone, comprising 65% of T_4's weight, and 58% of T_3's. The thyroid hormones are the only iodine-containing compounds with established physiologic significance in vertebrates. Beside the thyroid, other glands have the capacity of concentrating iodine such as salivary glands, gastric mucosa, mammary glands, and choroid plexus (Bernard *et al.*, 2004).

The oceans are the primary source of iodine in the world. The concentration of iodine in the sea water is a bout 50-60 μg/liter, about the same as in human serum. Seaweeds are thought to have an important role in the biogeochemistry of iodine, they can take up iodine and concentrate it in their tissues. It was estimated that seaweeds known as the kelp (*Laminaria digitata)* can contain as much as thirty thousand times more iodine than the surrounding seawater (Gall *et al.*, 2004).

The average iodine concentration in the earth is 300μg/kg, and in the air is about 0.7μg/m^3 (Fleicher *et al.*, 1974). Iodine in the sea and air are the dominant for the iodine cycle in the nature.

2.1.1 Iodine cycle

Iodine evaporates from the ocean and enters the atmosphere, it can become attached to dust particles or precipitated in rainfall. It then enriches the soil and returns to the ocean via rivers.

Contributions to iodine also originate from human animal sources (Vought, 1972). Iodide ions can be oxidized by sun light to volatile elements iodine [Miyake *et al.,* 1963]. The atmospheric iodine concentration is usually less than 0.7 μg/m^3, but it constitutes an important part of the iodine cycle. (Egri *et al.*, 2006). About 14-20 μg of iodine can be added to the daily iodine intake, since "standard man" breathes 20 m^3 of air per day (Koutras *et al.,* 1980).

Rain contain much more iodine (1.8-8.5 µg iodine/liter) than does the air (Duce *et al.,* 1963) in this way rain enriches the superficial layers of soil with iodine.
Heavy rain on sloping ground may wash away the superficial iodine-rich layers, where as the newly exposed soils are iodine poor, thus endemic goitre areas are more likely to be found in regions with soil erosion (Malamos *et al.,* 1971).

Inland regions far from the ocean have the greatest risk of iodine deficiency, mountains areas have the most serious iodine deficiency such as in Alps, Jabal Marra, and Himalayas, where iodine in the soil has been washed by rain and glaciers (Mastorakos *et al.,* 2006). However, iodine deficiency is observed in regions of low altitude such as central Africa, central Asia, and part of central Europe. Iodine deficiency is also associated with areas exposed to frequent flooding and in large river deltas such as those of the Ganges, yellow river, and Rihn (Eltom 1984, Dunn 1990).

There is no general figure of iodine concentration valid for any particular material, except for sea water. Iodine concentration of drinking water correlates inversely with endemic goitre prevalence (Koutras *et al.*, 1980). The iodine content of terrestrial plants varies considerably depending on the iodine content of the soil and the water in the area (Koutras *et al.*, 1980). Brown algae such as kelps and wracks (seaweed) are generally very high in iodine content, constituting up to 0.7 % of the wet plant weight. A dose of 1 g of kelp daily is said to provide the 0.1 to 0.2 mg of iodine required by a normal adult (Indergaard & Minsaas, 1991).

Iodine content of most of the terrestrial plants is rather low, averaging 1µg/kg dry weight, except Spanish which contains about 200 µg iodine /kg. Plankton contains high concentration of iodine (Shacklette *et al.,* 1967).

The iodine content in animals depends on the concentration of this element in the plant on which they feed and the iodine content of meat is generally higher than that of vegetables (Koutras *et al.,* 1980).

The iodine content of food varies according to the plant season and the way of food processing. Higher values of iodine in milk during winter, and lower during summer, have been reported (Broadhead *et al.,* 1965). Iodine is added to the food, and it is also lost by cooking, it has been observed that, the iodine content of fish was reduced 29% by frying, 23% by grilling and 58% by boiling (Harrison *et al.,* 1965). The iodine content of food stuff from different regions and countries depend on the natural iodine content as well as iodine supplements in the form of iodine salt, iodine-enriched animal's feeds, iodine containing veterinary medicinals, sanitizing agents and coloring substances (Koutras *et al.,* 1980).

Dietary iodine is absorbed in the small intestine in the form of iodides, these are loosely bound with proteins and are carried by the blood to the thyroid gland.

About one third of iodide is selectively absorbed by the thyroid (Williams, 1986). Inorganic iodide enters the thyroid follicular cells and is transformed through a series of metabolic steps into the thyroid hormones thyroxin (T_4) and 3,5,3 triiodothyronine.

2.1.2 Chemical Nature and Excretion of thyroid Iodine

The greatest part of iodine in the thyroid glands exists in the form of thyroglobulin. Thyroglobulin is unique among body proteins in its content of iodinated amino acids (Taurog 1978). The iodotyrosine "T_4 and T_3 precursor" are the most abundant iodinated amino acid components of thyroglobulin. Purified thyroglobulin varies in total iodine content depending on iodine intake.

Inorganic iodide represents only small fraction of the total iodide, and it probably exerts important regulatory effects on thyroid function (Taurog 1978). A small percentage of thyroid iodide is present in the form of iodinated amino acids. Two thirds of the absorbed iodine is excreted in the urine within 2 to 3 days after

ingestion, and may also be lost in the faeces, sweat and milk. Most of this iodine comes from breakdown of thyroid hormones (Williams 1986).

2.1.3 Physiological function of iodine

Iodine participates in the synthesis of the thyroid hormones as it's the only known function in human metabolism. The thyroid hormones, thyroxin T_4 and triiodothyronine T_3 stimulate cell oxidation and regulates basal metabolic rate, apparently by increasing the oxygen uptake and reaction rate of the enzymes handling glucose. Thus thyroid hormones are involved in many different ways in human metabolism and are essential for normal growth and mental and physical development. In this role iodine indirectly exerts tremendous influence in the body's over all total metabolisms (Williams 1986).

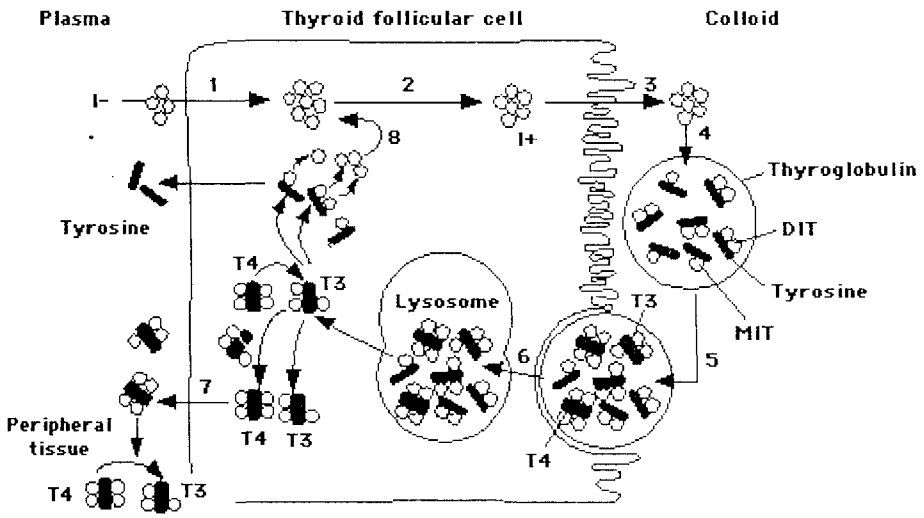
2.1.4 Iodine metabolism and thyroid hormones synthesis

Iodine enters the thyroid follicle cells as inorganic iodide and is transformed through a series of metabolic steps into the thyroid hormones T_4 and T_3 (Fig. 2.1). The major external influence on this system is thyrotropin (TSH). The Na^+/I^- symporter mediates the first and key step in the process of supplying iodide to the gland in transporting iodide against the electrochemical gradient across the *thyroid's basal membrane into the cytoplasm of the follicular cells (Carrasco,* 2000). Besides the inorganic iodide transported from the serum into the thyroid, some iodide derives also from deiodination of organic iodine compounds within the gland. Iodide must first be oxidized to a higher oxidation state before it can act as an effective iodinating agent.

Only H_2O_2 is sufficiently potent to oxidize iodide (Corvilain *et al.,* 1991). At the apical membrane thyroid peroxidase (TPO) catalyzes the iodination of tyrosyl residues of thyroglobulin (Tg) producing either monoiodotyrosine (MIT) or diiodotyrosine (DIT) (Taurog, 1970). The iodination process involves H_2O_2,

iodide, thyroperoxidase (TPO), and glycosylated Tg. Two residues of diiodotyrosine then couple within Tg to form T_4, or one diiodotyrosine and one monoiodotyrosine to form T_3. This coupling reaction is also catalyzed by TPO (Taurog, 2000). The mature iodinated Tg molecule is stored in the colloid. About one-third of Tg's iodine is in T_4 and T_3, the remainder being in the inactive precursors, monoiodotyrosine and diiodotyrosine (Dunn & Dunn, 2000). Prior to secretion from the thyroid, T_4 and T_3 must be released from peptide linkage within Tg. Tg retrieved by icropinocytosis passes first through the endosome system, where proteolysis and hormone release is initiated, then into lysosomes, where the process is completed (Dunn & Dunn, 2000). T_4 is the main secretory product of the thyroid and is then deiodinated to its biologically active metabolite T_3. Seventy to 90% of the daily production of T_3 originates from extrathyroidal deiodination from T_4, with the rest derived from the thyroid. Not all internalized Tg undergoes proteolysis. Some is recycled back to the follicular lumen, apparently by a selective process targeting immature Tg molecules (Dunn & Dunn, 2001).

Fig. (2.1): Summary diagram of major steps in thyroid hormone biosynthesis



Thyroid hormone biosynthesis Thyroid hormone synthesis includes the following steps: (1) iodide (I^-)trapping by the thyroid follicular cells; (2) diffusion of iodide to the apex of the cells; (3) transport of iodide into the colloid; (4) oxidation of inorganic iodide to iodine and incorporation of iodine into tyrosine residues within thyroglobulin molecules in the colloid; (5) combination of two diiodotyrosine (DIT) molecules to form tetraiodothyronine (thyroxine, T4) or of monoiodotyrosine (MIT) with DIT to form triiodothyronine (T3); (6) uptake of thyroglobulin from the colloid into the follicular cell by endocytosis, fusion of the thyroglobulin with a lysosome, and proteolysis and release of T4, T3, DIT, and MIT; (7) release of T4 and T3 into the circulation; and (8) deiodination of DIT and MIT to yield tyrosine. T3 is also formed from monodeiodination of T4 in the thyroid and in peripheral tissues. (Modified from Scientific American Medicine, Scientific American, New York, 1995.)

2.1.5 Iodine intake

The daily iodine intake of adult humans varies from less than 10 µg in areas of extreme deficiency to several hundred milligrams for some persons receiving medicinal iodine. Milk, meat, vitamin preparations, medicines, radiocontrast material, and skin antiseptics are important sources (Dunn, 1994). The daily iodine intakes recommended by WHO, (UNICEF) and (ICCIDD) (WHO *et al.*, 2001) for different life stages is shown in table (2.1).

Table 2.1: WHO recommended daily intake: optimal iodine nutrition

Population sub-group	Amount
Adults	150 µg/day
Pregnancy and Lactation	200 µg/day
Children (6-12 years)	120 µg/day
Infants (0-5 years)	90 µg/day

Daily iodine intake recommended by WHO, UNICEF and ICCIDD (WHO *et al.*, 2001) for lactating women is based on the assumption that an increment of 50 µg/day is needed to cover the daily iodine requirement of the infant (National Research Council, 1989) resulting in a recommended daily iodine intake of 200 µg since the recommended daily iodine intake for infants is 50 µg (WHO *et al.*, 2001). Studies have shown that elevated TSH concentration is one of the first effects of iodine excess (Roti & Vagenakis, 2000). Although an elevated TSH concentration may not be a clinically significant adverse effect, it is an indicator for increased risk of developing clinical hypothyroidism and was therefore chosen as the critical adverse effect on which to base the Tolerable Upper Intake Level for iodine (Institute of Medicine, 2002).

Too much iodine increases the incidence of iodine-induced hyperthyroidism, autoimmune thyroid disease and perhaps thyroid cancer. Too little causes mental retardation, goitre, hypothyroidism, and other features of the so-called iodine deficiency disorders (Dunn, 1998).

Although goitre is the most visible indicator, iodine deficiency produces a spectrum of disorders that are termed the iodine deficiency disorders (IDD) (Hetzel, 1983). These include goitre, hypothyroidism, cretinism, congenital anomalies, neurological dysfunction, impaired reproduction, still birth and spontaneous abortion. Iodine is essential for the human body, as it is part of the thyroid hormones thyroxin (T_4) and triiodothyronine (T_3). These hormones are

involved in many different ways in human metabolism and are essential for normal growth and mental and physical development.

2.2 Iodine metabolism in iodine deficiency

When iodine intake is abnormally low, adequate secretion of thyroid hormones may still be achieved by marked modifications of thyroid activity (Delange, 2000b). Iodine deficiency leads to increased TSH stimulation, increased iodine uptake, rapid iodine turnover, and enhanced production of T_3 relative to T_4. However, the response of rats to iodine-deficient diets can be markedly affected by the strain of rat and by nutritional factors other than the iodine content of the diet (Okamura *et al.*, 1981). These results suggest that both hereditary and nutritional factors may be involved in the variable responses of humans to iodine deficiency.

2.2.1 Alterations in the thyroid metabolism

Serum T_4 concentration was greatly reduced in rats fed iodine-deficient diets, and most of the T_3 in the circulation arises directly from the thyroid. This occurs not only through increased thyroidal biosynthesis of T_3, but also through deiodination of T_4 by the greatly increased levels of deiodinase in the activated gland (Pazos-Moura *et al.*, 1991). The shift to increased T_3 secretion and serum T_3:T_4 ratios may play an important role in the adaptation to iodine deficiency because T_3 is the most active thyroid hormone and requires less iodine for synthesis (Greer *et al.*, 1968). Similarly, it has been shown that the monoiodotyrosine/diiodotyrosine ratio is increased in iodine deficiency (Ermans, *et al.*, 1963a). However, insufficient iodination of Tg appear to be responsible for reduced efficiency of thyroid hormone synthesis (Dumont *et al.*, 1995).

2.2.2 Increase in iodine uptake

The most important adaptation of the thyroid to an insufficient iodine supply is to increase the trapping of iodine. The accumulation in the thyroid of about 100 µg per day must be ensured (Delange, 2000b). To preserve existing iodine stores, the amount of iodine excreted in the urine must be reduced to a level corresponding to the level of iodine intake. A linear proportionality between iodine excretion and iodine intake within the physiological range has been shown by Vought & London (1967).

As long as the iodine intake remains above a threshold of about 50 µg/day, the absolute uptake of iodide by the thyroid remains normal and the organic iodine content of the thyroid remains within the limits of normal (i.e. 10-20 mg), despite a decrease in the serum iodine concentration (Delange, 2000b).

2.2.3 Thyroid enlargement

The thyroid gland has a unique structure and is the largest of the organs that functions exclusively as an endocrine gland (Capen, 2000). The basic unit of cellular organization in the mature thyroid is the thyroid follicle. This consists of a lumen filled with viscous colloid and is surrounded by a single layer of epithelial cells enclosed by a basement membrane (Pintar, 2000). The basic process in the transformation of the normal thyroid to a goitrous gland is the generation of new thyrocytes and follicles (hyperplasia) in addition to increasing cell volume (hypertrophy). Besides TSH, other thyroid growth-stimulatory factors are thought to be of importance in the increased follicular cell replication. Association between elevated TSH levels and thyroid enlargement are not consistent. In rats, it has been shown that during goitre development TSH mainly induces hypertrophy, whereas intracellular iodine content mainly regulates thyroid hyperplasia (Stubner *et al.*, 1987). Whether TSH stimulation or intrathyroidal iodine depletion is more important for thyroid growth is difficult to determine (Pisarev & Gartner, 2000) and probably depends on the severity of iodine deficiency.

Whereas in the early days goitre was considered as an adaptation to iodine deficiency, there is now no doubt that the large colloid goitre is a maladaptation (Delange *et al.*, 2001). Theoretically, the optimal thyroid response to iodine deficiency would be an increase in thyroid blood flow, in iodide trapping capacity and in iodination rate, and rather low Tg content in a much reduced colloid space (Dumont *et al.*, 1995). However, in endemic goitre the thyroid gland is often large and filled with colloid. The low iodine and the high Tg concentration lead to a lesser iodination of Tg. Increased hydrolysis of large amounts of this protein is necessary to achieve normal secretion.

Excessive hydrolysis and deiodination of released iodotyrosines floods the thyrocyte iodide compartment and results in a leak of iodide (Ermans *et al.*, 1963b). In consequence, the urinary iodine (UI) loss will be enhanced and lead to an aggravation of iodine deficiency creating a vicious cycle with further dilution of luminal iodide versus Tg (Dumont *et al.*, 1995). Accordingly, the ideally adapted thyroid would grow by a factor of no more than 2, and be comprised of an increased number of small follicles.

2.3 Role of thyrotropin on the function of the thyroid gland

TSH is the primary factor that regulates the function of thyroid follicular cells and, ultimately, thyroid hormone secretion. In a classic negative feedback system, thyroid hormone inhibits the synthesis of TSH directly at the pituitary level and indirectly at the hypothalamic level by reducing the secretion of thyrotropin-releasing-hormone (TRH) (Cohen *et al.*, 2000). Elevated serum TSH levels have been reported in humans with chronic iodine deficiency (Delange *et al.*, 1971, Chopra *et al.*, 1975). It has been suggested that the iodine-deficient thyroid is more sensitive to TSH (Bray 1968), but the biochemical mechanism for this increased sensitivity are unknown (Pisarev & Gartner, 2000). The lack of systematic correlation between goitre and TSH levels indicates that differences in

the duration of elevated TSH levels and in thyroid responsiveness to TSH, as well as other factors, may determine whether goitre develops (Dumont *et al.*, 1992).

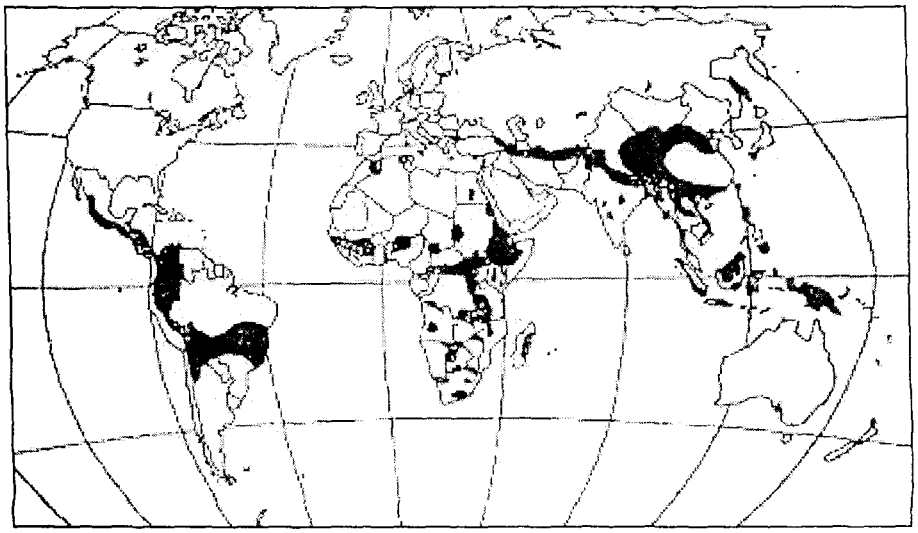
2.4 Iodine deficiency disorder (IDD)

Iodine deficiency disorders (IDD) is the collective name of endemic goitre and endemic cretinism. It is a major worldwide problem, especially during pregnancy and childhood (Dunn & Delange 2001). It is a threat to the social and economic development of countries. The most devastating outcomes of iodine deficiency are increased perinatal mortality and mental retardation. Iodine deficiency is the main preventable cause of brain damage in children and constitutes a universal public-health concern (Andersson *et al.*, 2005). This was the primary motivation in the current worldwide drive to eliminate IDD (Andersson *et al.*, 2005). Although cretinism is the most extreme manifestation, the more subtle degrees of mental impairment leading to poor school performance, reduced intellectual ability and impaired work capacity are of considerably greater significance (Andersson *et al.*, 2005). It has been estimated in 1990 that 1.6 billion people "that is 28.9% of the earth's population" were at risk of iodine deficiency, which therefore appeared as the world greatest single cause of preventable brain damage and mental retardation (Delange *et al.*, 2001). In 1999, the World Health Organization (WHO) estimated that 13% of the world's total population was affected by goitre (WHO *et al.*, 2001).

The populations at risk are those living in an environment where the soil has been deprived of iodine. This arises from the distant past through glaciations, compounded by the leaching effects of snow, water and heavy rainfall, which removes iodine from the soil (Mastorakos *et al.*, 2006). Most of the mountainous districts in the world have been or still are endemic goitre regions. These include Andes, Himalayas, the Alps, Greece and the Middle Eastern countries, in many

foci in the People's Republic of China, and in the highlands of New Guinea. There are also important endemias in non mountainous regions, as the belt extending from the Cameroon grasslands across northern Zaire and the Central African Republic to the borders of Uganda and Rwanda, Holland, Central Europe and the interior of Brazil. An endemic existed in the Great Lakes region in North America two generations ago, the soil of flooded river valleys in the Ganges Valley in India. Measurements have indicated that these regions have in common a low concentration of environmental iodine. The iodine content of the drinking water is low, as is the quantity of iodide excreted each day by residents of these districts. The deficiency in the soil leads to iodine deficiency in all forms of plant life and cereal grown in the soil. Hence populations living in systems of subsistence agriculture are "locked into" iodine deficiency (Koutras *et al.,* 1980). It is recently estimated (Andersson *et al.*, 2005) that the iodine intake of 36.5% (285 million) school-age children worldwide is insufficient. In South-East Asia, 96 million children were iodine deficient, in Africa and the Western Pacific an estimated 50 million children were suffering from iodine deficiency. The total number of children with iodine deficiency in Europe and the Eastern Mediterranean are about 40 million children each, and in the Americas 10 million. The highest proportions of iodine-deficient children are found in Europe (59.9%) and South-East Asia (39.9%) while the lowest are found in the Western Pacific (26.2%) and the Americas (10.1%). Extrapolating from the proportion of school-age children to the general population, it is estimated that nearly two billion individuals have insufficient iodine intake Worldwide.

Map shows the world wild distribution of iodine deficiency disorders in the developing countries (ACC/SCN 1987c)



2.4.1 Iodine deficiency in the fetus

Iodine deficiency is considered the leading cause of preventable mental retardation (Delange *et al.*, 2001). Severe iodine deficiency during pregnancy can lead to endemic cretinism in the offspring, which is the most serious IDD. Two types of cretinism have been described: 1) neurological cretinism: marked by dominant neurological disorders, extreme mental retardation and a high prevalence of deafmutism, and 2) myxedematous cretinism: marked by severe thyroid insufficiency (Dumont *et al.*, 1994). Many intermediate forms exist between these two forms of cretinism (Dumont *et al.*, 1994, Delange 2000a). However, endemic cretinism only constitutes the extreme expression of a spectrum of abnormalities in physical and intellectual development and in the functional capacities of the thyroid gland (Delange 2000a). The degree of severity of iodine deficiency that takes place during pregnancy determines the potential harmful effects on the fetus (Glinoer 2001). Effects of less severe iodine deficiency during pregnancy on cognitive function later in life are difficult to determine as many

confounding factors can complicate the interpretation of the results. However, some studies showed clear evidence of the adverse effects of iodine deficiency during pregnancy. Fierro-Benitez *et al.*, (1988) compared 8 and 15 year old school children of mothers who had received iodized oil during pregnancy to children of a neighboring comparable community whose mothers had not received iodized oil. Statistical significant differences in tests of intellectual function were not found, but results showed distinct differences in maturation of psychomotor function between the two groups. In a case-control study in Bangladesh, comparing mental retardation according to maternal history of goitre, found an increased risk of reduced intelligent scores in children of goitrous mothers (Durkin *et al.*, 2000). Haddow *et al.*, (1999) tested the neuropsychological development of children whose mothers were hypothyroid during pregnancy. Although none of the children were hypothyroid as newborns, their full-scale intelligence quotient scores at the age of 7 to 9 years were 7 points lower than those of the matched controls. These results indicate that maternal hypothyroidism has adverse effects on the child's development even without immediate clinical manifestation. As thyroid hormones are transferred from mother to fetus, both before and probably even after the onset of fetal thyroid function (Glinoer & Delange, 2000), maternal thyroid sufficiency might therefore be most important in early pregnancy.

2.4.2 Iodine deficiency in the neonate and during infancy

In contrast with adult data, which have shown that the iodine stores of the thyroid are not affected by iodine deficiency unless severe iodine deficiency is present, iodine content in the thyroid of newborns depletes with milder iodine deficiency (Delange, 2000b). Alterations of thyroid function in newborns have been reported from less severe endemic areas, even when thyroid function in adults was normal (Sava *et al.*, 1984). Therefore, newborns are particularly sensitive to the effect of iodine deficiency. The most important effect of iodine deficiency on the brain takes place during fetal life and early infancy at the time of maximum growth rate

of the brain (Hetzel 1994). However, it is difficult to distinguish between the effects of gestational iodine deficiency and postnatal iodine deficiency responsible for any observed intellectual deficits. In a study in China, the effects of iodine supplementation during pregnancy and early life were studied and compared to older children who had not previously received iodine. Children treated prenatally had fewer neurological abnormalities, increased head growth, and an improved developmental quotient compared to children who were treated during neonatal period (Cao *et al.,* 1994). Compared with untreated children, iodine supplementation during the third trimester and during the newborn period was associated with a trend toward higher development scores, although it did not improve neurological development (Cao *et al.,* 1994).

2.4.3 Iodine deficiency in childhood and adulthood

Iodine deficiency in childhood and adulthood causes goitre. Although some studies have shown larger thyroid volumes (Tvol) in girls than in boys (Elnagar *et al.,* 1995; Djokomoeljanto *et al.,* 2001), others have found no gender difference in Tvol (Elnour *et al.,* 2000; Xu *et al.,* 1999). A meta-analysis of 18 studies on mental development in endemic goitre areas (17 severe and 1 mild) showed that non-cretin and clinically euthyroid individuals had a mean loss of 13.5 intelligence quotient points compared to controls from nearby iodine-sufficient areas after correction of iodine deficiency by iodized oil (Bleichrodt & Born, 1994). However, most children growing up in an iodine-deficient region were also exposed to iodine deficiency during fetal life. Therefore, it is not known to what extent lasting effects of maternal iodine deficiency are responsible for any intellectual deficits and what effect individual iodine deficiency during childhood contributes. Studies that examined to what extent the damage of mild iodine deficiency on the cognitive function is reversible have reported controversial results. A randomized iodine supplementation trial among goitrous children (5-12 y) in Bolivia observed no significant change in intelligent scores (Bautista *et al.,*

1982). In contrast, in school children in Malawi iodine supplementation significantly improved mental and psychomotor performance (Shrestha, 1994). Sudan is also reported among the countries affected by the consequences of the IDD (Eltom *et al.,* 1984, Elnagar *et al.,* 1995, Bani, 2006). In a goitre study conducted in the village of Soja located in Darfur state, where the prevalence of goitre was found to be 79.6%, with severe iodine deficiency, a primary school in the village had to be closed by the educational authorities in 1975 mainly because of failure of almost all of the pupils to pass the examinations for entry to the secondary schools (Elnagar *et al.,* 1995).

Beside the public health impact, iodine deficiency has also an adverse economic effect. As hypothyroid people move more slowly, think less clearly, require more sleep, and respond sluggishly to stimuli compared to euthyroid people, they are less efficient in many tasks (Dunn, 1994). This can impair significantly work productivity, and consequently, can handicap the economy by reducing work output.

2.5 Endemic Goitre

The term endemic goitre is a descriptive diagnosis and reserved for a disorder characterized by enlargement of the thyroid gland in a significantly large fraction of a population group. Goitre is considered endemic in a population when more than 5% of the preadolescent (6-12) school-age children have enlarged thyroid glands as reported by WHO, UNICEF and International Council for Control of Iodine Deficiency Disorders (ICCIDD), (WHO *et al.,* 2001). Based on the observation that goitre prevalence of 5% may be associated with a range of abnormalities including inadequate urinary iodine excretion (UIE) or subnormal levels among adults, children and neonates (Zimmermann *et al.,* 2003). The main etiological factor of endemic goitre is the insufficiency of iodine in the daily diet.

Since nontoxic goitre also exists when there is abundant iodine in the diet, the distinction between endemic and non endemic goitre is necessarily arbitrary. WHO recommends that iodine deficiency surveys examine school-age children (6-12 years) because of their high physiologic vulnerability and their accessibility through school for studies in baseline health parameters (Zimmermann *et al.*, 2004). The WHO cutoff points applied for classifying iodine nutrition into different degrees of public health significance are shown in (Table 2. 2).

Table 2.2 Epidemiological criteria for assessing iodine nutrition based on median urinary iodine concentrations in school-aged children (WHO *et al.*, 2001).

Median urinary iodine (µg/L)	**Iodine intake**	**Iodine nutrition**
<20	Insufficient	Severe iodine deficiency
20-49	Insufficient	*Moderate iodine deficiency*
50-99	Insufficient	Mild *iodine deficiency*
100-199	Adequate	Optimal
200-299	More than adequate	Risk of iodine-induced hyperthyroidism in susceptible groups
>300	Excessive	Risk of adverse health consequences (iodine-induced hyperthyroidism, autoimmune thyroid disease)

2.6 Etiology of Goitre

Several studies have documented the epidemiological link between insufficient iodine intake and the development of endemic goitre. Iodine deficient areas are mainly characterized by soil from which iodine has been leached by glaciations,

heavy rainfall and flood (Hetzel 1993). Populations in these areas depending on food locally grown, consequently get iodine-deficient. However, the greater availability of methods assessing iodine deficiency has demonstrated that IDD occur in many areas where none of these conditions were found (WHO *et al.*, 2001). The fact that significant iodine deficiency has been found in regions where IDD have been considered to be eliminated by prophylactic programs (WHO *et al.*, 2001), support the assumption that other nutritional factors may influence the prevalence and severity of iodine deficiency. Besides goitrogenic foods, other nutritional interactions, such as protein-energy malnutrition (Gaitan *et al.*, 1983) and micronutrient deficiencies (Boyages, 1993, Zimmermann & Kohrl, 2002) may counteract the response to iodine prophylaxis.

2.7 Goitrogenic factors

Agents that cause thyroid enlargement are known as goitrogens. They may cause goitre by acting directly on the thyroid gland, or indirectly by altering the regulatory mechanisms of the thyroid gland and the peripheral metabolism and excretion of thyroid hormones (Gaitan, 1990). Potential goitrogenic substances or their precursors are widespread in vegetables of the Brassica family (Fenwick & Heaney, 1983). Naturally occurring goitrogens cyanoglucosides in several staple foods such as cassava, maize, bamboo shoots, sweet potatoes, and lima beans are more effective goitrogens (Gaitan, 1990). It has been reported (Delange, 1982) that large differences in glucosides content of plants belonging to the same family and the same taxonomy and grown within the same geographical area owing to their genetic backgrounds and ecological factors. It is also reported that (Oke, 1982) the goitrogenic or antithyroid potential of a plant not only depends on the relative concentrations of cyanogenic constituents found in fresh plant but also on its processing as food, so the common measures to reduce the goitrogenic potency include soaking, washing, boiling, cooking *etc.* After ingestion, these glucosides release cyanide, which is detoxified to thiocyanate. Thiocyanate is a powerful

goitrogenic substance as it is an anion with the same molecular size as iodine. It inhibits thyroid accumulation of iodide and, at higher doses, competes with iodide during it is binding (Wollman, 1962). Thiocyanate has been shown to compete with iodine, as it serves as a substrate for TPO and therefore inhibits the iodination of tyrosyl residues of Tg (Michot *et al.*, 1980). In contrast, thiocyanate stimulates the coupling reaction (Virion *et al.*, 1980). As iodine also is a substrate for the Tg iodination and a stimulatory ligand for the coupling reaction, suggesting that thiocyanate binds to the same regulatory site as iodine but has a slightly different affinity (Michot *et al.*, 1980). As reviewed by Delange (2000b), several studies have shown that cassava plays a role in the etiology of endemic goitre together with iodine deficiency (Thilly *et al.*, 1993, Zimmerman 2004). In a recent goitre prevalence and iodine nutritional status study among school children conducted in West Bengal in India (Chandra *et al.*, 2006) consumption of cyanogenic plant foods in the region as evidenced from the urinary excretion of thiocyanate (SCN) was the major etiological factor in elevated goitre prevalence (38%) in spite of adequate iodine intake. It was also reported that 17-19 Indian cyanogenic plant foods that are used as common vegetables have potent anti-thyroid activity and supplementation of extra iodine even fails to counteract their effect (Chandra, 2004).

Cassava is confirmed as a potent goitrogenic factor determined by the concentration of thiocyanate in the urine of individuals and the ratio between dietary iodine intake and thiocyanate (Thilly *et al.*, 1993, Chandra *et al.*, 2006). Experimental and epidemiological studies have shown that thiocyanate overload aggravates the severity of iodine deficiency and worsens its outcome (Vanderpas *et al.*, 1984, Delange 1989, and Contempre *et al.*, 2004). However, the common association of these two factors in Central Africa is not sufficient to explain the more restricted prevalence of myxedematous cretinism (Levander, 1992). Other staple foods also contain goitrogens such as phenolic compounds released from millet and babassu, which is a staple food in Brazil (Gaitan *et al.*, 1995). TPO

activity was confirmed to be inhibited by these phenolic compounds (Gaitan *et al.*, 1989). The regular consumption of these staple foods may contribute to the genesis of endemic goitre in areas of iodine deficiency. Millet diets rich in C-glycosylflavones (C-GF) which are goitrogenic. Three well defined goitrogenic C-GF compounds were isolated from millet (Gaitan *et al.*, 1989) these are glycosylvitexin, vitexin, and glycosylorientin. In epidemiological study in the severe endemic goitre region of Darfur in western Sudan (Moreno-Reyes *et al.*, 1993) a significantly higher prevalence of goitre and hypothyroidism were detected among children fully weaned and fed porridge prepared with millet and cow's fat compared with children exclusively breast fed. The study was considered additional evidence supporting the concept that C-GF and thiocyanate in millet play a role in goitrogenesis and endemic juvenile hypothyroidism, since the urinary iodine concentration was uniformly low and did not vary significantly with age. In vivo antithyroid effects of vitexin in rats (Gaitan *et al.*, 1995) the acute oral administration of purified vitexin to rats receiving the iodine-rich diet produced distinct and significant inhibition of the coupling mechanism in the thyroid. The coupling mechanism was demonstrated by a high ^{125}I-labeled monoiodotyrosine (MIT) and diiodotyrosine (DIT) over ^{251}I-labeled T_3 and T_4 ratio and low ^{125}I-labeled T_3 and T_4 concentrations. It was also reported by the authors that, Vitexin produced distinct inhibition of TPO-catalyzed protein iodination in a dose-response manner which indicated that vitexin is a potent antithyroid drug does inhibit TPO and, consequently, is a potential goitrogen.

2.7.1 Goitrogenic factors in drinking water

Water has special significance in different body homeostasis because of its large consumption and better uptake metabolism of trace elements from their ionic state in water. Monitoring of water sources and drinking water for different chemical elements has become very important (Ahmed *et al.*, 2004). It was early introduced

by Hellwig (1935) that calcium with a low iodine intake produced a hyperplasic thyroid and that a slight increase in iodine intake caused a colloid goitre.

In experimental study (George *et al.*, 1942) rats fed three calcium salts, calcium chloride, lactate and phosphate to determine whether calcium could be classed as a goitrogenic substance. When other factors are kept constant, all of the evidence indicates that calcium does not influence the size of the thyroid gland. However, along with vitamin D only calcium chloride can act as a goitrogenic agent. While the mechanism of this action cannot be absolutely determined from these experiments, the results suggest that chloride causes some loss of iodine which is followed by an increase in thyroid weight when an excess of calcium is absorbed.

Hibbard's (1933) also observed that calcium chloride salt was found to produce goitre. However, Hibbard's concluded that chloride is not goitrogenic supported by these results since without vitamin D, calcium chloride did not cause an increase in thyroid weight.

The disinfection of potable water with chlorine-based disinfectants represents the most dominant class of inorganic xenobiotics ingested continuously (Bercz and Garner1991). The chlorine-based disinfectants have been reported occasionally to affect the endocrine homeostasis and thyroid hormone levels in blood in animals (Konova *et al.*, 1999).

In a morphometric study in lambs (Konova *et al.*, 1999) consumption of drinking water containing 0.3 mg/L chlorine and the long-term consumption of water with 1.8 mg/L were associated with a significant increase in follicular epithelial cell height and a significant frequency distribution shift to the higher height categories without detectable macroscopical thyroid changes. The authors suggested antithyroidal effects of chlorine or chlorinated products formed when chlorine reacts with organic matter in the upper gastrointestinal tract. It has also been shown (Bercz *et al.*, 1982) that decreased serum thyroxin levels occur in monkeys and neonatal rats during subchronic exposure to chlorine dioxide in drinking water.

Fluoride was firstly prescribed by doctors to reduce the activity of the thyroid gland for those suffering from hyperthyroidism (Stecher, 1960; Waldbott, 1978). Water fluoridation increases thyroid-depressing medication leading to higher levels of hypothyroidism in the population, and all the subsequent problems related to this disorder. Fluoride exposure in fluoridated communities is estimated to range from 1.6 to 6.6 mg/day, which is a range that overlaps the dose (2.3 - 4.5 mg/day) shown to decrease the functioning of the human thyroid (Galletti & Joyet, 1958). In Russia, Bachinskii (1985) found a lowering of thyroid function among healthy people, at 2.3 ppm fluoride in water. It has been estimated that (Monson, 1990) in humans effects on thyroid function were associated with fluoride exposures of 0.05–0.13 mg/kg/day when iodine intake was adequate and 0.01–0.03 mg/kg/day when iodine intake was inadequate. It has been calculated by Manson (1990) that for a 70-kg person (standard man), fluoride doses as low as 3.5 mg/day for those with an adequate intake of iodine, and 0.7 mg/day for those with an inadequate intake of iodine may have an affect on the thyroid. For the most susceptible people with iodine deficiency the total dose of fluoride from all sources should be less than 0.7 mg/day. It has been recommended that the fluoride in drinking water should be zero when the daily intake of fluoride in the diet already exceeds this dose (WHO, 2003).

Excess fluoride was reported as a major cause of goitre endemicity in the Northwest part of South Africa (Steyn, 1955). The same findings were repeated by Jooste in 1999 that, endemic goitre in the absence of iodine deficiency in schoolchildren of the Northern Cape Province (Jooste, 1999). A similar relationship between excess fluoride exposure and goitre has been reported in England as well as in the Punjab region of India (Wilson, 1941), in Nepal (Day and Powell-Jackson, 1972), and in Gujarat, India (Desai, 1993). Other studies, however, have failed to find this relationship (Burgi, 1984).

2.8 Assessment of iodine deficiency

2.8.1 Target group

It has been recommended by the WHO (WHO *et al.*, 1994) that in the assessment of iodine status in a region, selection of a population group the following criteria should be considered: vulnerability, representativeness and accessibility.

Applying these criteria, the most useful target groups are school-age children because of their high vulnerability and easy access. Moreover, school-age children are often affected by other health concerns such as other micronutrient deficiencies. However, a major drawback of school-based surveys in developing countries is that children not attending school are not represented. This possibly leads to biased prevalence estimates (WHO *et al.*, 2001).

2.8.2 Urinary iodine

When the need for thyroidal iodine has been met, excess iodine is excreted by the kidney (Hetzel 1993). About 90% of iodine intake within the physiological range is eventually excreted in the urine (Vought & London 1967). The median urinary iodine excretion (UIE) in casual samples is currently the most practical indicator to assess recent dietary iodine intake. It is important to consider that iodine excretion of individuals varies over the day and between days, but this variation can be evened out by a big enough sample size. Therefore, the iodine concentration in spot urine samples of children and adults provides an adequate assessment of a population's iodine nutrition (WHO *et al.*, 2001).

2.8.3 Thyroid size

The other indicator for assessing the extent of iodine deficiency in a population is the prevalence of goitre (WHO *et al.*, 1994). For years, palpation has been the single method available for defining thyroid volume (Tvol). In 1994, a new two-grade classification system was proposed by WHO/UNICEF/ICCIDD. It defined

goitre as any enlarged thyroid that is palpable (Grade 1) or visible (Grade 2) (WHO *et al.*, 1994). This simplified the use of palpation even more. However, in areas of mild IDD where the prevalence of visible goitre is low, sensitivity and specificity of palpation are poor, and misclassification can be as high as 40% (WHO *et al.*, 1994; Andersson *et al.*, 2005). Under these conditions ultrasonography is more reliable. The higher sensitivity of ultrasonography becomes even more important when the impact of iodine prophylaxis is monitored, as the Tvol are expected to decrease over time. It is a safe, non-invasive specialized technique. Using portable ultrasound equipment, it can be performed in the field and, using a generator, even under conditions without electric current. In a workshop organized by WHO and ICCIDD where interobserver and interequipment variation in ultrasound Tvol was evaluated, it was suggested that a systematic bias of one examiner has led to an overestimation of the current reference criteria (Zimmermann *et a.*, 2000d).

Besides the debate on Tvol reference criteria during the last few years, there is also a controversy on the usefulness of Tvol as an indicator in determining the impact of universal salt iodization programs (USI). Little is known on how long it takes for the goitre to disappear or if thyroid enlargement is completely reversible at all. In rats, iodine supplementation abolishes not only hypertrophy, but also hyperplasia of the glands and restores normal function and regulation (Stubner *et al.*, 1987). In school children, Tvol by ultrasound had not changed 395 days after treatment with 120, 240, 480 mg of oral iodized oil, whereas in the groups receiving 960 mg oral iodine or an intramuscular injection of 480 mg iodine Tvol was decreased by –29% and –23%, respectively (Benmiloud *et al.*, 1994). Jooste *et al.*, (2000) found 1 year after mandatory iodization of salt in South Africa no difference in goitre rate by palpation in school children.

On the other hand, a number of goitre cross sectional studies revealed that iodine supplementation had marked effects on goitre reduction. In a study in schoolgirls in Omdurman, a single dose of oral iodized oil capsules containing 200 mg iodine

result in a significant reduction in the goitre size and/or total disappearance of goitre in some girls (Abdel Monim *et al.,* 1993).

Thirteen months after oral administration of potassium iodide solution, 8.7 mg every second week or 29.7 mg every month, mean Tvol measured by ultrasound had decreased in both groups to values comparable with those in iodine-sufficient areas (Todd & Dunn, 1998). However, there is not much data on the effect of iodized salt on the reduction of thyroid enlargement. In a randomized trial in school children in China, Tvol by ultrasound decreased to normal after 18 months of salt iodized at 25 ppm (Zhao *et al.,* 1999).

It is now recognized that once Universal Salt Iodization (USI) is launched, the prevalence of low UI will fall faster than the prevalence of goitre (Sullivan & May, 1999). After some period when USI has been achieved, the prevalence of low UI and goitre will again be in agreement indicating no IDD. Until then, the use of Tvol as an indicator might be of limited value. However, it is unknown how long this adaptation takes. Therefore, it is important that the results on thyroid size be interpreted cautiously to judge the success of USI (Sullivan & May, 1999) unless iodized salt has been available for a long period.

2.8.4 Blood constituents

Determining serum concentrations of the thyroid hormones, T_4 and T_3, is usually not recommended for monitoring iodine nutrition (WHO *et al.,* 2001). It is argued that, even though in iodine deficiency, serum T_4 is typically lower and serum T_3 is higher than in normal population, the overlap is large enough to make these tests not practical for epidemiological studies. However, the aim of any USI is the prevention of adverse effects of iodine deficiency. Normalization of the thyroid function is therefore the major goal and its assessment is advisable.

Similar recommendations are given for TSH concentration. As the difference between iodine-deficient and iodine-sufficient population groups is neither great nor consistent, much overlap occurs between individual TSH values. Therefore,

the blood TSH concentration in school-age children and adults is not a practical marker for iodine deficiency, and its routine use in school-based surveys is not recommended (WHO *et al.*, 2001). However, neonatal TSH screening is considered very useful in assessing IDD status of a population. An elevated TSH level in neonates and infants is of concern because it indicates inadequate thyroid hormone concentration during the crucial stage of brain development. Consequently, TSH concentrations reflect the risk of damage to the developing brain and subsequent impairment of intellectual development (Delange *et al.*, 2001). However, it is only recommended as an indicator for iodine deficiency if a national program already exists (WHO *et al.*, 2001).

Another blood constituent which can serve as surveillance indicator is thyroglobulin (Tg). Tg is the most abundant protein of the thyroid and provides a matrix for the synthesis of the thyroid hormones and a vehicle for the subsequent storage (Dunn & Dunn 2000). A small amount of Tg is secreted into the blood circulation by a mechanism which is still unclear (Chopra & Sabatino 2000). Abnormal serum Tg concentrations result from abnormalities in Tvol, excess thyroidal stimulation, or physical thyroid damage (Spencer, 2000). Tg rises in individuals with an insufficient iodine intake and it normalizes before Tvol has increased (WHO *et al.*, 1994). Tg has been shown to correlate well with other indicators of iodine deficiency (Missler *et al.*, 1994, Briel *et al.*, 2001, Zimmerman *et al.*, 2006). However, a major limitation to the use of Tg in IDD monitoring are assay-dependent factors influencing Tg measurement reliability which include lack of a standard reference material, poor sensitivity of some assays, and poor interassay precision (Torrens & Burch, 2001, Zimmerman *et al.*, 2006). In IDD control programs, different indicators were used to evaluate the suitability of indicators of iodine status and thyroid function in schoolchildren in iodine-deficient area of Benin (Tina *et al.*, 2001) Tg, TSH, FT_4 in serum, thyroid volume and urinary iodine concentration were measured. Initial concentrations of TSH and FT_4 were within the normal range in the total studied population groups, whereas

rum Tg, urinary iodine concentration and thyroid volume were indicative of oderate-to-severe iodine deficiency. Ten months after oral iodized iodine supply, rum Tg was significantly decreased and urinary iodine concentrations gnificantly increased in the supplemented group compared with nsupplemented one. This indicated that serum Tg and urinary iodine ncentrations are the indicators mostly affected by the iodine supply. The authors gued that the normal reference ranges of serum concentrations of TSH and FT_4 e too wide for detecting iodine deficiency in schoolchildren age.

Iodization programs

Iodized oil

o main strategies to correct iodine deficiency are iodine supplementation rtification. Iodization of salt, irrigation water, drinking water and bread are nt possibilities to fortify with iodine (Burgi & Helbling, 1996). DeLong *et* 997) showed that iodine supplementation of irrigation water of wheat in as of severe iodine deficiency decreases neonatal and infant mortality. There s a successful experimental study on fortification of sugar in small area in Kosti *own central* Sudan (Eltom *et al.,* 1994). However, besides salt fortification neither f the other strategies has been used in large scale. Although salt fortification has een the ultimate goal (WHO *et al.*, 1999), the use of iodized oil is recommended ıen immediate iodine supplementation is needed during the implementation of SI.

e most frequently used iodized oil is Lipidol, a seed-oil from the opium poppy, *hich* iodine atoms are bound to the polyunsaturated fatty acids (Ingenbleek *et , 1997).* A portion of the iodized fatty acids is stored in adipose tissue (Wei & 1985), permitting a slow release of iodine and thus providing long-lasting plies. One year of iodine needs can be achieved with 200 to 480 mg in the form ›ral Lipidol (Benmiloud *et al.*, 1994; Elnagar *et al.*, 1995). The advantage of iodized oil is that it can be selectively applied to circumscribed regions or

geographical pockets of severe iodine deficiency, within such a region, it can be restricted to certain target populations to reduce costs (Abdel Monim 1993, Burgi & Helbling 1996).

2.9.2 Universal salt iodization

In nearly all countries where iodine deficiency occurs, it is now well recognized that the most effective way to eliminate IDD is through USI (WHO *et al.*, 2001). Salt is an ideal vehicle for fortification due to the following reasons: (1) It is consumed by everyone, (2) The consumption is rather constant throughout the year, (3) Its production is usually limited to a few centers which facilitates its quality control, (4) Salt iodization is easy to implement and is cost-effective, (5) the addition of iodine to the salt does not change color and flavor. The recommended amounts for the daily intake of iodine is 150 µg/day for adults and adolescents, 200 µg/day for pregnant and lactating women and less for children (WHO *et al.*, 2001). In order to achieve an optimal iodine intake through salt iodization the following factors have to be considered: 1- The consumption of salt per person, 2- The degree of iodine deficiency, 3- The iodine losses during storage and transport. Consequently, the optimal level of salt iodization varies from country to another (Delange *et al.*, 2001). However, WHO/UNICEF/ICCIDD recommend that iodine concentration should be 20-40 mg/kg salt in typical circumstances, where the average daily salt intake is 10 g per person, 20% of iodine from salt is estimated to be lost during transport from production to household and 20% during cooking (WHO *et al.*, 1996).

While there is much data on the effects of the health benefits of iodized oil, there is a lack of such data on iodized salt. However, long-term effects of USI are well known. Burgi *et al.*, (1996) has reviewed the effects of iodized salt in Switzerland, which has first started the introduction of iodized salt in 1922. After 1930 no new born endemic cretins have been identified, and goitre disappeared rapidly in newborns and school children, more slowly in army recruits, and

incompletely in elderly adults. In Finland iodized salt was introduced in the 1940's. Consequently the goitre prevalence among school children decreased generally to 1-4%, having been 15-30% in most parts in the early 1950's (Lamberg *et al.*, 1981). However, results on the elimination of endemic cretinism, the prevention of blunting of intellectual and socioeconomic potential and reduction in perinatal morbidity and mortality through salt iodization are needed (Delange *et al.*, 2001).

2.9.2.1 Monitoring universal salt iodization

There has been a remarkable progress in USI worldwide. In 1999, out of the 130 countries with IDD, 98 had legislation on salt iodization in place (WHO *et al.*, 1999). However, the past has shown that once a national IDD control program is successfully implemented, monitoring is very important to maintain sustainability. If iodine content in the salt is too low, iodine deficiency will relapse soon (Delange *et al.*, 2001). At the same time, it is crucial to avoid iodine excess as this can lead to adverse effects. The principle adverse effect is iodine-induced hyperthyroidism which occurs essentially in older people with autonomous nodular goitre, especially when iodine intake is suddenly too much increased (Delange *et al.*, 1999). In this case, excess iodine can even have lethal consequences for some individuals. However, according to Delange & Lecomte (2000) the incidence of this disorder is usually low and reverts spontaneously to the background rate of hyperthyroidism or even below this rate after 1 to 10 years of iodine fortification. Iodine-induced hyperthyroidism can not be entirely avoided even when iodization programs use only physiological amounts of iodine (Delange & Lecomte, 2000). It is very important to introduce USI at the lowest iodine level to correct IDD and at the same time to minimize the risk of iodine-induced hyperthyroidism. Moreover, it is crucial to maintain the iodization level in the salt at the recommended level. In a global Cross-sectional data on urinary iodine and total goitre prevalence in school-age children from 1993–2003 (Andersson *et al.*,

2005) Forty-three countries have reached optimal iodine nutrition global wise. Strengthened UI monitoring is required to ensure that salt iodization is having the desired impact, to identify at-risk populations and to ensure sustainable prevention and control of iodine deficiency. Efforts to eliminate iodine deficiency should be maintained and expanded (Andersson *et al.*, 2005).

2.10 Interactions between iodine and other micronutrients and thyroid metabolism

Micronutrient deficiencies represent a largely invisible ('hidden hunger') but often devastating form of malnutrition that is particularly prevalent in developing countries (Bani 2006). More than 3 billion persons worldwide are afflicted by vitamin A deficiency, iron deficiency, or iodine-deficiency disorders (Ash *et al.*, 2003).

Deficiencies of vitamin A, iron, and iodine often occur simultaneously because of 4 underlying factors including poverty limits food choices, unfavorable ecology combined with seasonal cycles limits the availability of micronutrient-rich food sources, nutrients interact synergistically, and absorption and metabolism are influenced by total diet quality; thus, a deficiency of one nutrient may lead to a deficiency of another, and finally, parasitic infections may cause blood loss, reduced appetite, or decreased absorption of nutrients, thereby adversely affecting micronutrient status (Ash *et al.*, 2003).

Together, these 3 micronutrient deficiencies constitute a devastating public health problem that greatly contributes to the cycle of underdevelopment and hinders the attainment of education, health, and productivity goals in countries throughout the world. More than 2 billion people in the world today are estimated to be deficient in key vitamins and minerals, particularly vitamin A, iodine, iron and zinc. Most of these people live in low income countries and are typically deficient in more than one micronutrient (WHO *et al.*, 2001). The known effects of micronutrient deficiencies include impaired physical and mental growth among children, iron

deficiency anaemia, maternal mortality, low adult labour productivity and blindness (Zimmermann *et al.,* 2006). Table 2.3 shows the etiology and vulnerable groups for iodine, iron, vitamin A and selenium deficiencies. Micronutrient deficiencies co-exist and interact in different ways.). Biassoni (1998) in his study explain the reason why some individuals exposed to the same conditions of iodine deficiency develop goitre while others do not. Iodine deficiency is a permissive condition, but other nutritional and/or environmental conditions are instrumental. These may favor the induction of an enlargement of the thyroid gland or protect an individual from the effects caused by iodine deficiency (Biassoni *et al.,* 1998). It is now recommended by the WHO (WHO *et al.,* 2001) to consider supplementation and fortification using multiple micronutrients. Beside preventing more than one deficiency at a time, multiple supplementation strategies could have additional benefits due to interactions between two or more micronutrient deficiencies. Some micronutrients beside iodine are essential for normal thyroid metabolism. Of these iron, selenium, vitamin A and zinc. Deficiencies of iron and selenium can act in concert with iodine deficiency to impair thyroid metabolism and modify the response to prophylactic iodine (Arthur *et al.,* 1999, Zimmermann & Köhrle 2002). The effects of iron and selenium status on iodine and thyroid metabolism share certain parallels (Zimmermann & Köhrle 2002).

Table 2.3: Etiology and vulnerable groups for iodine, iron, vitamin A and selenium deficiencies (WHO *et al.,* 2001)

	Iodine deficiency	**Iron deficiency**	**Vit-A deficiency**	**Sel-deficiency**
Etiology	Geographic	Dietary Increased losses	Dietary Increased losses	Geographic
Vulnerable groups	Entire population	Pregnant and lactating women Infants Preschool children Women of childbearing age	Pregnant and lactating women Infants of less than 6 months old Preschool children	Entire population

CHAPTER THREE

SUBJECTS AND METHODS

3.1 Study description

This study is a descriptive cross-sectional study. The survey took place in the period from June to November 2006.

The study was approved by the health and education authorities, school nutrition as well as from local governmental head quarters in the selected cities. Written approval was also obtained from the student's activity agency belonging to the Ministry of Education of Khartoum State.

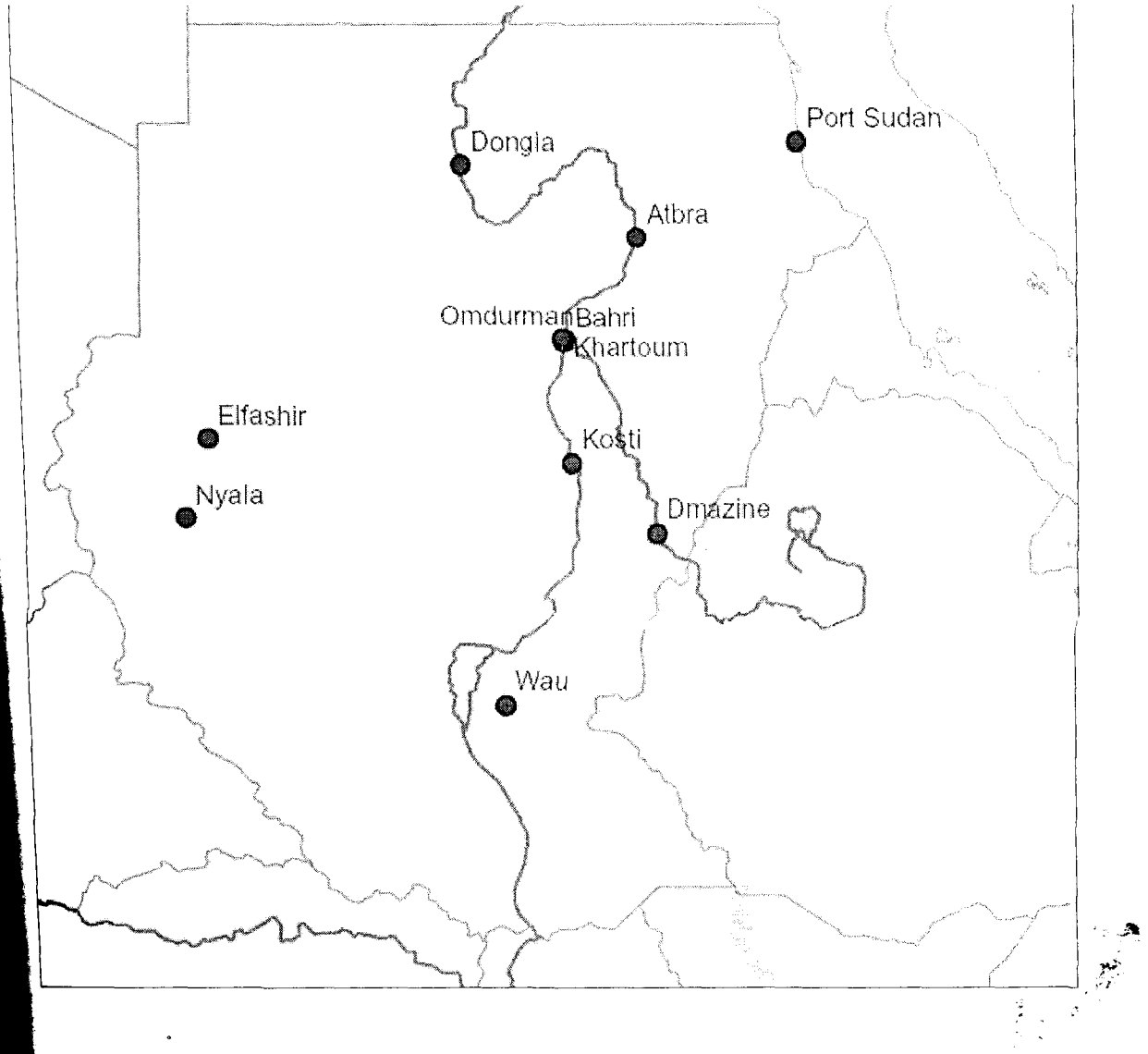
The aim of the study was explained to local administrative and traditional authorities and teachers. Written approvals from local authorities, the parents and the parents-teachers association were obtained. All children selected were examined by the same investigator. No child was excluded on health grounds.

3.2 Study area

Goiter prevalence was measured in schoolchildren from selected preliminary schools in nine Sudanese cities which are the capitals and the biggest cities of their states. These include Nyala, Elfasher, Wau, Atbara, Kosti, Dongula, Dmazine, Port Sudan and Khartoum as it is shown in Fig. 3.1.

Khartoum was considered as three different towns Khartoum, Khartoum North and Omdurman according to it's large population. It was estimated by IDD that six millions out of 32 millions "the total population of Sudan" were living in Khartoum (IDD, 2006).

Fig. 3.1: Map of Sudan showing the cities included in this study



3.3 Subjects

The subjects of this study were male and female schoolchildren of 6 to 12 years old from the cities selected.

3.4 Sample size and collection

The sample size was determined as 379 children for each city. Sample selection was obtained by random sampling technique (Kevin, *et al.*, 1995). Selection of the schools to be participated in the survey was based on dividing every town into three sectors. One school from each sector was randomly selected regardless of the gender. The list of schools was obtained from the local educational authority. The prevalence of goitre was measured in all pupils in the junior classes (from the first to the 4^{th} class). Three children were selected from each class using systematic random sampling technique for the blood and urine samples collection. The number of students in the class was divided by 3 to determine the sample interval (X) in each class. Using the simple random technique a random number was selected within the sample interval as starting point. (X) was added to the number of the first child to select the second child in the sample. The unwilling pupils were substituted randomly by another one from the same class.

A round 150 to 200 children who attended the selected primary schools on the days of the survey, were studied for the presence of goiter. In each city 479 to 659 children were clinically assessed for the presence of goiter. In this way a total of 6,181 pupils were clinically examined for the enlargement of thyroid gland. The age of the students was rounded-off to the nearest whole number.

In each class, children were assembled and briefed about the IDD survey activities. All the children in this study were examined for the presence of goiter using the palpation method, by the same investigator. Goiter size was graded according to the criteria recommended by the WHO/UNICEF/ICCIDD (1994) (grade 0, no goiter; grade 1, thyroid palpable but not visible; and grade 2, thyroid

visible with neck in normal position). The sum of grades I and II goiter provided the total goitre prevalence in the study population.

3.4.1 Blood sampling

Five ml venous blood samples were collected from selected children from each school irrespective of their thyroid status or gender from the clinically examined enrolled students.

Blood samples were collected from 10 to 12 children from each school, (30 to 36 children from each city). The samples were kept around 2 hours at room temperature, then serum samples were separated from the blood using bench centrifuge at 3000 RPM. Serum samples were collected and frozen at -20 C until analyzed. The samples were analyzed for the concentration of T_4, T_3, TSH, and Tg using sensitive radioimmunoassay methods.

3.4.2 Urine sampling

Casual urine samples (in 60 ml wide mouth screw capped plastic bottles) were collected from the same pupils from whom blood was collected. Portions of urine samples were kept at room temperature to be analyzed for iodine concentration. Another portion of the urine samples (10 ml) were kept at -20C to be analyzed for thiocyanate concentration.

Urinary iodine execration was measured using modified Sandell-Kothoff reaction (Pino, 1996). The reaction depends on the catalytic action of iodide ion the reduction of the ceric ion to the cerous ion in the presence of arsenious acid. The intensity of color formed was measured by using spectrophotometer. Known concentration range of iodine standards were measured with each batch of test samples. Because of unexpected high UI values, additional standards of higher concentrations were introduced.

Thiocyanate concentration was measured using method described by Aldridge (1945) as modified by Michajlovskij and Langer (1985) that depend on treatment

of the thiocyanate in the urine with trichloroacetic acid, followed by saturated bromine water and arsenious trioxide and allowed to react with pyridine-benzidine hydrochloride mixture. The intensity of colour formed was measured by using spectrophotometer. Thiocyanate standards were prepared from their respective potassium salts.

3.4.3 Water sampling

Water samples "in 600 ml screw capped plastic bottles" were collected from each school. The samples were collected from the drinking water resources available for the pupils. Water samples were analyzed for the concentration of Calcium, Magnesium, Chloride, Fluoride and total Hardness (Calcium carbonate concentration).

3.4.4 Determination of thyroid related hormones and Tg

All the thyroid related hormones, T4, T3, TSH and Tg were measured using radioimmunoassay (RIA) techniques. The reagents for these hormones were obtained from China Institute for Atomic Energy (CIAE) Department of Isotopes (Beijing/ China). The reagents included tracer, standards, antibodies, separating agents and quality control samples for different hormones.

The principle of RIA was described by Mardell in 1978 which sumerised that antigen (Ag) and labeled Antigen or tracer (Ag*) compete for a limited amount of antibody's (Ab) binding sites to form Ag-Ab or Ab- Ag* complexes. Counting the radioactivity of the bound complexes using Lithium Iodide crystal gamma counter. Construction of standard curve, the concentration of the antigen present in the sample can then be calculated. The principle of RIA technique can be illustrated in the following equation:-

Ag + Ab + Ag* → Ab-Ag + Ab- Ag* (complex) +Ag + Ag* → Appropriate separation system was applied to enhance fine separation of unreacted Ag and Ag* from the bound complexes.

Supernatant containing Ag, Ag* can be eliminated from the reaction tube by the aid of suitable separation system. The deposit containing (Ab-Ag +Ab- Ag*) can be counted in a suitable gamma counter.

All assay tubes were set in duplicates using the required protocols: proper mixing after each addition. All the assays were carried out using standard set and quality control sample (QCS), non specific binding (with no antibody) and total count with each batch.

The methods used for measurement of serum total thyroid hormones (T_4 and T_3) and thyroglobulin (Tg) were based on radioimmunoassay (RIA) technique, while TSH was measured using appropriate Immunoradiometric (IRMA) technique.

3.4.5 Determination of the chemical elements in water samples

Ca and Mg ions in water samples were measured using EDTA (Ethylenediaminetetraacetic acid) titrimetric method (Clesceri et al 2000 a) which depends on addition of Eriochrome Black or Calmagite dyes to an aqueous solution containing Ca and Mg at PH 10.0 $\pm$ 0.1 after addition of EDTA as chelating titrant.

Chloride ion was measured using Argentometric method (Clesceri et al 2000 b) that depends on titration of chloride with silver nitrate using potassium chromate for detection of end point. The reaction took place in a neural or slightly alkaline medium.

Fluoride was measured by SPADNS colorimetric method (Clesceri et al 2000 c) which based on the reaction of fluoride with zirconium-dye lake to form colorless complex anion $(ZrF_6)2^-$

CHAPTER FOUR

RESULTS

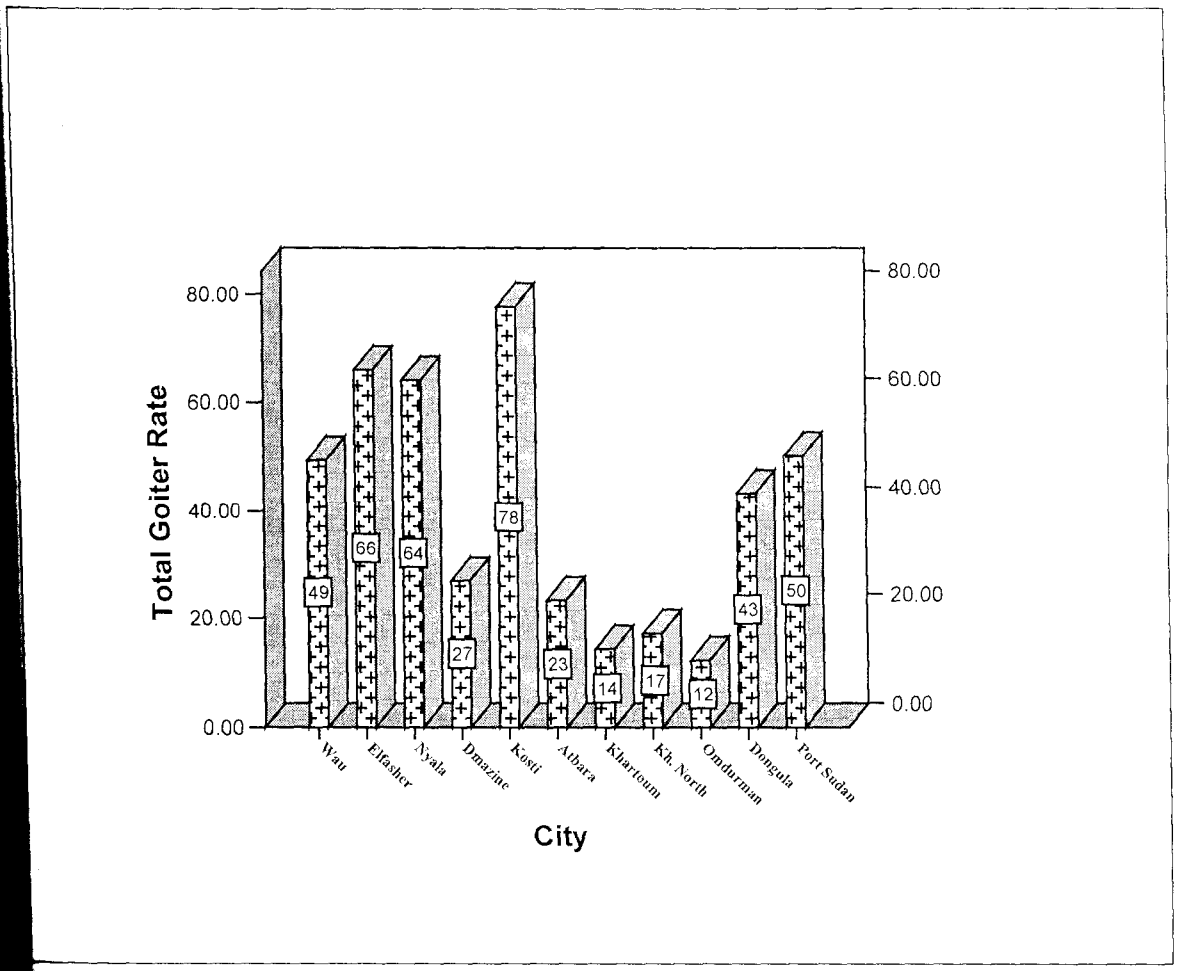
4.1 Goitre prevalence

Out of 6118 schoolchildren from 9 studied cities 2485 were found to be goitrous yielding total goitre rate (TGR) of 40.62%. TGR for each city is described in Table (4.1).

Table 4.1: Goitre prevalence in studied Sudanese cities

City	Goitrous	Non goitrous	Total	Percentage
Wau	255	261	516	49.42 %
Elfasher	435	224	659	66.01 %
Nyala	307	172	479	64.09 %
Dmazine	140	382	520	26.9 %
Kosti	407	117	524	77.67 %
Atbara	150	495	645	23.26 %
Khartoum	71	426	497	14.29 %
Khartoum North	89	430	519	17.15 %
Omdurman	72	517	589	12.22 %
Dongula	207	272	479	43.22 %
Port Sudan	328	326	654	50.15 %

Fig. 4.1: Total goitre rate (%) in studied Sudanese cities



4.2 Urinary iodine excretion (UIE)

The UIE as shown in Table (4.2) is indicating iodine deficiency from mild to severe in the whole study population except in Port Sudan in which UIE was reflecting sufficient iodine intake in all participating children. Samples below the limits of detection were assigned a value of 0.02 µg/dl. Fig. (4.2) shows the urinary iodine status. According to the WHO/UNICEF/ICCIDD criteria, severe iodine deficiency (UIE less than 2 µg/dl) was detected in 50 children out of 360 (13.89%), moderate iodine deficiency (UIE 2 to 4.9 µg/dl) in 78 (21.67%) and mild deficiency (UIE less than 10 µg/dl) in 125 children (34.72%). The overall median UIE was 6.55 µg/dl ranging from 0.02 to 73.6 µg/dl. The UIE results reflect iodine deficiency in 70.28% of the total study population, of these 128 children (35.56%) were excreting less than 5 µg/dl iodine in their urine indicating that iodine deficiency is a major national health problem. Fig. (4.3) and Table (4.3) show the median and range of the UIE for each city.

The UIE status in each city was as follows:-

Nyala: -

The median UIE was 6.15 µg/dl. In nine pupils out of 33 (27.27%) the UIE was higher than 15 µg/dl (the concentration of the highest standard) indicating that those students received iodine capsules recently distributed by UNICEF (just one month before the collection of urine samples for this study) through the local nutritional health authority. Severe iodine deficiency was detected in six of the pupils (18.18%). Moderate deficiency observed in seven (21.21%), while mild deficiency was found in 11 pupils (33.3%). In this city, 39.39% were found to excrete less than 5 µg of iodine in 100 ml of their urine.

Elfasher: -

The median UIE was 3.35 µg/dl. Only five pupils out of 34 (14.7%) had sufficient urinary iodine secretion. Severe iodine deficiency was indicated in six pupils (17.65%). One-half of the pupils secreted 2-4.9 µg/dl. Mild iodine deficiency was detected in 6 students (17.65%) also.

Dmazine: -

The median UIE was 7.30 µg/dl. Sufficient UIE was found in 6 out of 30 pupils (20%), the other 80% of the students had different degree of iodine deficiency.

Wau:-

The median UIE in this city was 9.40 µg/dl. Out of 37 pupils, 15 were found to have adequate UIE, while the majority of the pupils were iodine deficient.

Atbara:- The median UIE was 2.80 µg/dl. Only 5 pupils out of 35 had sufficient UIE. Iodine deficiency was detected in more than 85% of the pupils in this city.

Kosti:-

There was iodine deficiency in the whole population. The median UIE was 2.70 µg/dl. None of the pupils had reached the WHO cut off point (10 µg /dl) for the UIE. Furthermore, more than 96% of the pupils excreted less than 5 µg of iodine in 100 ml of their urine. Severe iodine deficiency was indicated in 12 subjects out of 32 (37.5%) who had UIE of less than 2 µg/dl. Only one student excreted 6 µg/dl iodine in urine. Thus, urinary iodine excretion in Kosti indicates severe iodine deficiency leading to the highest prevalence of goitre in the country as a whole.

Khartoum:-

The median UIE in Khartoum was 5.80 µg/dl. Only two pupils out of 30 (6.66%) had sufficient UIE of $\geq$ 10 µg/dl. Ten students (33.3%) had UIE ranging between 2 and 4.9 µg/dl, while the rest of the pupils (around 60%) with UIE of 5 to 9.9 µg/dl. None of the pupils had UIE of less than 2 µg/dl.

Khartoum North:-

The median UIE was 7.00 µg/dl. Out of 33 pupils 11 (33.3%) were found to have sufficient UIE, Ten of these pupils were living in the same area and belonging to the same school indicating iodine sufficiency in this area. Severe iodine deficiency was detected in 6 of the pupils (18.19%). Moderate Iodine deficiency was detected in two (6.06%). Mild iodine deficiency was observed in around 52% of the population.

Omdurman:-

The median UIE was 6.85 µg/dl. Severe iodine deficiency was detected in 4 pupils out of 33 (12.12%). Seven pupils (21.21%) showed moderate iodine deficiency. Mild iodine deficiency was detected in 11 (33.3%), while iodine sufficiency was indicated in one third of the population (33.3%) (Table 4.3).

Dongula:-

The median UIE was 8.90 µg/dl. Twenty out of 31 subjects (64.52%) were found to excrete 5 to 9.9 µg/dl iodine in their urine. Sufficient iodine intake was observed in 9 pupils (29.03%). Only two subjects (6.45%) were found to excrete between 2 to 4.9 µg iodine in their urine. Severe iodine deficiency was not detected.

Port Sudan:-

The UIE of the all population of this city indicated high sufficiency of iodine intake in the different parts of the city. The median UIE was 46.40 µg/dl with a range of 10.90 – 3.6 µg/dl (Table 4.3).

Table 4.2: Mean ±SD of serum T4, T3, TSH, TG, and urinary excretion of iodine and SCN of the study population

City	T_4 nm/l (Mean±SD)	T_3 nm/l (Mean±SD)	TSH mu/l (Mean±SD)	TG ng/ml (Mean±SD)	UIE µg/dl (Mean±SD)	USCN mg/dl (Mean±SD)
Nyala n=32	112.78± 22.83	1.90±0.40	1.83±1.04	23.3±11.2	7.60±6.12	0.45±0.29
Elfasher n=34	110.05± 19.62	1.70±0.35	1.73±0.86	28.3±11.0	4.81±3.87	0.45±0.2
Dmazine n=33	104.64± 25.56	1.97±0.43	2.71±1.39	30.9±21.5	7.92±2.78	0.45±0.18
Wau n=37	112.38± 34.23	2.22±0.73	1.69±0.86	25.4±15.3	10.15±5.31	0.48±0.17
Atbara n=35	108.68± 26.22	1.85±0.60	2.42±1.09	40.5±13.6	4.24±4.26	0.41±0.14
Kosti n=32	110.37± 26.54	1.74±0.40	1.78±1.07	67.0±25.3	2.86±2.17	0.36±0.15
Khartoum n=30	101.00± 30.84	2.13±0.46	1.71±0.85	56.3±15.5	6.09±2.49	0.34±0.11
Khartoum North n=33	96.79± 25.65	2.19±0.41	2.18±1.77	38.5±21.6	8.94±6.40	0.36±0.13
Omdurman n=32	105.34± 29.14	2.19±0.35	1.95±0.84	38.0±22.5	7.80±4.58	0.35±0.12
Dongula n=31	87.42± 23.12	1.84±0.48	3.13±1.81	27.2±10.1	9.49±4.29	0.31±0.11
Port Sudan n=31	82.58± 27.93	1.54±0.49	3.71±3.10	46.0±23.4	45.29±18.82	0.29±0.11
Total n=360	103.23± 28.15	1.94±0.52	2.25±1.58	38.0±22.1	10.30±13.02	0.39±0.17

4.3 Urinary thiocyanate excretion (USCN)

Table (4.2) shows the mean ± STD of urinary thiocyanate (USCN) in each city as well as the overall mean of the study population. The median and the range are shown in Tale (4.3) and Fig. (4.4). The highest mean value of USCN was found in Wau city (0.48±0.17 mg/dl) while the lowest value was detected in Port Sudan (0.29±0.11 mg/dl). The overall mean of the USCN of this study was (0.39±0.17), while the overall median was 0.37 mg/dl with a range of 0.05 to 1.25 mg/dl.

According to the cut off point established for the USCN excretion which is 0.46 mg/dl, Out of 360 pupils 105 (29.17%) were found to excrete high levels of SCN in their urine (Fig. 4.5).

4.4 Serum samples

The overall Mean ± STD of serum TSH, T_3 and T_4 concentrations are shown in Table (4.2). The median and range of these hormones are shown in table (4.3). The overall mean serum TSH, T_3 and T_4 concentrations were within the normal reference range according to the estimation of Sudan Atomic Energy Commission which reported that total serum T_4 is 60 -165 nmole/liter, serum T_3 is 0.8 to 3.0 nmole/liter, serum TSH is 0.7 to 5.0 MU/l. The normal range for serum thyroglobulin according to the kit's producer (Beijing atom high-tech co., LTD, Beijing / China) is 4.1 to 24.0 ng/ml. There is no normal range or cutoff point reported in any of Sudanese institutions. The overall mean serum T_4 in this study (103.23± 22.10 nmole/l) while the overall median was 102.00 with a range 0f 41.0-198 nmole/l. Figure (4.6) shows the median and the range of T_4 in each city. Comparing T_4 concentrations, the mean serum T_4 in Port Sudan and Dongula were significantly lower than in the other cities (Pvalue <0.05), Fig. (4.6). In this study, biochemical hypo or hyperthyrodism is only considered when it is confirmed by at least two thyroid related hormones.

4.4.1 Serum T_4

Figure (4.7) describes the status of serum T4 according to the normal range estimated by Sudan Atomic Energy Commission (SAEC). Five of the pupils (1.39%) were found to be hypothyroid confirmed by their low serum concentration of T_4 (< 60 nmole/L) and elevated serum concentration of TSH (>5.0 MU/L). Hyperthyroidism was confirmed in 7 of the pupils (1.94%) who had high serum concentrations of T_4 (>165 nmole/L) and low serum concentrations of TSH (<0.7

MU/L). Serum T_4 was low in 14 pupils with normal T_3 and TSH levels. Figure (4.6) shows the median and range of the serum T_4 levels in each city

4.4.2 Serum T_3

Table (4.3) and Fig. (4.8) show the median and range of the serum T_3 levels in each city. The overall median serum T_3 was 1.90 nmole/l with range of 0.60-4.60 nmole/l. According to the SAEC normal range for T_3 which is 0.8 to 3.0 nmole/l, low T_3 concentration was found in 4 out of 360 children (1.1%) while elevated values were detected in 10 (2.8%), (Fig. 4.9). Low mean serum T_3 concentration was found in Port Sudan compared with mean values in the other city (P value <0.05), Fig. (4.8).

4.4.3 Serum TSH

Table (4.3) and Fig. (4.10) show the median and the range of serum TSH level in each city. Table (4.2) show the mean ± SD of serum TSH for each city as well as the overall mean serum TSH which was 2.35±1.58 mu/l. Serum TSH was high in 15 subjects (4.17%) and low in 8 (2.22%) when compared with the normal values estimated by SAEC (0.7 – 5.0 mu/l), (Fig. 4.11). Mean serum TSH concentration was higher in Port Sudan compared with mean values in the other city (P value <0.05), (Fig. 4.10).

4.4.4 Serum Tg

The mean serum Tg was 38.0±22.1 ng/ml (Table 4.2). Figure (4.12) and Table (4.3) show the median and the range of serum Tg in each city. The overall median and range of serum Tg was 34.10 mg/dl and 0.3-137.7 mg/dl. Figure (4.13) describes the serum Tg status according to the cutoff points estimated by the kit's producer. On the basis of the WHO criteria for serum Tg, which reported that, serum Tg level of 10 mg/dl is considered a minimum value for iodine intake sufficiency. While serum Tg levels of less than 10 mg/dl were taken as a marker of

iodine insufficiency. According to the WHO criteria, only 19 pupils out of 360 (5.28%) had normal serum Tg equal or less than 10 mg/dl, the cutoff point for sufficient iodine intake. According to the kit's producer, 101 children (28.06%) had normal serum Tg values of 24.5 mg/dl or less. High values of serum Tg (more than 24.5 mg/dl) were found in 259 children (71.94%), indicating that the iodine intake is in adequate in the vast majority of the studied population. Although the highest Tg mean concentration was found in Kosti 67.0±25.3 in which the median UIE was the lowest (2.70 µg/l) compared with the other cities values, there was a lack of correlation neither between serum Tg level and urinary iodine excretion, nor between serum Tg and any of the thyroid related hormones.

Correlation of TGR and thyroid parameters

As it is shown in Table (4.7), there were no significant correlation between total goitre rate (TGR) and other thyroid parameters (serum T_4, TSH, UIE and USCN). TGR is correlated negatively with serum T_3 ($P<0.000$). Serum T_4 is significantly proportional to the serum T_3 ($P<0.000$) and inversely proportional to the serum TSH ($P<0.004$), UIE ($P<0.001$) and USCN ($P<0.001$). There was no significant correlation between serum T_4 neither with serum Tg nor with the TGR. TSH is negatively correlated with the UIE ($P<0.001$). There was no correlation between serum TSH and TGR or with USCN. The UIE was significantly correlated with the serum TSH ($P<0.001$) and negatively with serum T_4 ($P<0.001$). There was no correlation between UIE with the Tg or USCN concentrations. The USCN concentration was correlated with serum T_4 ($P<0.001$) as well as with T_3 ($P<0.05$) and negatively correlated with serum Tg ($P<0.005$).

Table 4.3: Median values and (range) of serum T4, T3, TSH, TG, and UIE and USCN of the study population.

City	T_4 nm/l Median (Range)	T_3 nm/l Median (Range)	TSH mu/l Median (Range)	Tg ng/ml Median (Range)	UIE µg/dl Median (Range)	USCN mg/dl Median (Range)
Nyala n=32	112.50 (65.0-151)	1.90 (1.20-3.30)	1.50 (0.50-5.10)	20.9 (6.6-56.8)	6.15 (0.02-16.5)	0.48 (0.06-1.25)
Elfasher n=34	115.0 (73.0-142)	1.65 (1.10-2.70)	1.50 (0.70-4.20)	27.2 (11.3-51.9)	3.35 (0.06-16.4)	0.45 (0.11-1.07)
Dmazine n=33	103.0 (58.0-148)	2.10 (1.00-3.00)	2.20 (1.20-7.20)	27.1 (1.0-101.5)	7.30 (3.40-16.5)	0.41 (0.12-0.80)
Wau n=37	114.0 (54.0-191)	2.00 (1.20-4.60)	1.40 (0.40-3.90)	23.2 (1.0-70.1)	9.40 (0.02-20.0)	0.49 (0.09-0.82)
Atbara n=35	100.0 (42.0-162)	1.80 (0.60-3.90)	2.40 (0.80-4.70)	40.4 (10.1-77.7)	2.80 (0.02-14.3)	0.40 (0.18-0.72)
Kosti n=32	112.0 (41.0-161)	1.70 (1.0-2.60)	1.65 (0.20-4.70)	60.0 (37.9-137.7)	2.70 (0.02-8.50)	0.35 (0.12-0.78)
Khartoum n=30	98.5 (47.0-198)	2.05 (1.50-3.50)	1.5 (0.70-4.60)	55.0 (27.4-83.1)	5.80 (0.02-14.7)	0.32 (0.14-0.64)
Khartoum North n=33	92.0 (56.0-177)	2.20 (1.30-3.20)	1.70 (0.80-11.3)	36.8 (0.3-84.5)	7.00 (0.05-20.0)	0.37 (0.06-0.70)
Omdurman n=32	103.5 (50.0-182)	2.25 (1.40-2.80)	1.90 0.60-3.60)	39.6 (2.6-87.1)	6.85 (0.30-18.6)	0.34 (0.15-0.67)
Dongula n=31	81.0 (58.0-135)	1.80 (0.70-2.60)	2.60 (1.0-10.10)	27.8 (7.7-46.8)	8.90 (4.30-40.8)	0.31 (0.05-0.59)
Port Sudan n=31	78.0 (52.0-187)	1.60 (0.70-2.80)	3.20 (0.80-17.9)	49.9 (0.4-92.6)	46.40 (10.9-73.6)	0.26 (0.11-0.50)
Total n=360	102.00 (41.0-198.0)	1.90 (0.60-4.60)	1.80 (0.20-17.9)	34.1 (0.3-137.7)	6.55 (0.02-73.6)	0.37 (0.05-1.25)

4.5 Water samples

Table (4.4) shows Total Goitre Rate (TGR) and the concentration of fluoride, chloride, calcium, magnesium and the total hardness ($CaCO_3$) in the drinking-water collected from schools in the selected cities. The concentrations of the all elements are expressed as (mean ± SD) in mg/l. There were remarkable variations in the concentrations of the measured elements between cities as well as between samples within the same city indicating the variability of the water resources available for the pupils and the population of these cities. Water samples were collected from 3 different schools located in different parts of each city. The mean concentration of the elements in the water samples are shown in figs. 4.14-4.18.

4.6 Correlation of water chemicals with TGR and thyroid parameters

Table (4.6) shows the correlation between thyroid parameters, water chemical components as well as cross correlations between water components and thyroid parameters.

Although the mean concentration of the water chemical contents are nearly within the permissible concentrations reported by the WHO (WHO 1993, WHO 2006), there were strong correlations between these water contents and the level of thyroid hormones and the TGR shown in Table (4.6).

The total hardness of water is significantly correlated with TGR ($P<0.05$), serum TSH ($P<0.000$), and inversely proportional to the concentration of serum T_4 ($P<0.01$) and serum T_3 ($P<0.000$). The total hardness of water is also significantly correlated with the other water contents such as Ca ($P<0.000$), Mg ($P<0.000$) and Cl ($P<0.000$). Total hardness is also inversely correlated with fluoride concentration ($P<0.000$).

Cl content of the water is significantly proportional to the TGR ($P<0.000$), serum Tg ($P<0.005$), UIE ($P<0.01$), and negatively proportional to the serum T_3 concentration. There is also significant correlation of Cl with water hardness, Ca and Mg ($P<0.000$) and inverse relation with the F content ($P<0.05$).

Ca content of water is strongly correlated with the thyroid parameters (Table 4.5). It has significant correlation with serum TSH ($P<0.001$), UIE ($P<0.000$) and inversely correlated with serum T_3 ($P<0.000$) and serum T_4 ($P<0.005$). There is no correlation between Ca content of water and TGR.

The Fluoride concentration in the water samples is significantly correlated with UIE ($P<0.000$) and the serum Tg concentrations (Table 4.6). fluoride is significantly correlated with the other water chemical contents. There is an obvious but insignificant correlation between fluoride content in water and TGR ($P=0.055$).

Table 4.4 : Total goiter rate and average concentrations of chloride, fluorine, calcium, magnesium and hardness of drinking water in the studied area

City	TGR	chloride mg/l Mean±SD	fluoride mg/l Mean±SD	Calcium mg/l Mean±SD	magnesium mg/l Mean±SD	Hardness $CaC0_3$ mg/l Mean±SD
Nyala	64.09 %	15.75±1.26	0.79±0.46	38.65±7.97	19.64±17.14	177.49±67.91
Elfasher	66.01 %	30.78±8.68	0.63±0.16	68.11±24.17	26.60±18.95	320.94±69.48
Dmazine	26.9 %	17.51±0.34	1.31±0.12	38.86±6.89	20.80±3.68	182.60±31.30
Wau	49.42 %	15.63±3.76	1.25±0.27	13.03±4.91	7.52±3.05	52.83±20.77
Atbara	23.26 %	61.91±62.47	0.17±0.07	48.13±15.25	33.55±17.10	258.39±100.69
Kosti	77.67 %	18.35±2.98	0.83±0.14	15.02±0.46	9.15±5.96	75.20±24.16
Khartoum	14.29 %	21.53±10.08	0.25±0.09	38.96±18.07	16.78±9.30	166.47±82.48
Kh.North	17.15 %	7.83±1.57	0.77±0.02	38.95±4.86	15.08±7.25	159.50±36.65
Omdurman	12.22 %	14.16±4.70	1.09±0.16	25.04±9.75	11.37±7.87	109.64±53.16
Dongula	43.22 %	13.78±4.39	0.71±0.41	41.83±0.88	21.01±2.72	191.08±9.00
P. Sudan	50.15 %	247.72±74.6	1.00±0.13	73.90±26.58	52.79±5.47	402.03±82.29

Table 4.5 : Correlation of TGR to the chemical contents of the drinking water in the studied areas

Correlations

			CL mg/l	Ca mg/l	Mg mg/l	F mg/l	Hardness mg/l	Total Goiter Rate
Spearman's rho	CL mg/l	Correlation Coefficient	1.000	.434**	.502**	-.124*	.561**	.428**
		Sig. (2-tailed)	.	.000	.000	.018	.000	.000
		N	360	360	360	360	360	360
	Ca mg/l	Correlation Coefficient	.434**	1.000	.630**	-.296**	.910**	.055
		Sig. (2-tailed)	.000	.	.000	.000	.000	.297
		N	360	360	360	360	360	360
	Mg mg/l	Correlation Coefficient	.502**	.630**	1.000	-.019	.788**	.000
		Sig. (2-tailed)	.000	.000	.	.720	.000	1.000
		N	360	360	360	360	360	360
	F mg/l	Correlation Coefficient	-.124*	-.296**	-.019	1.000	-.200**	.101
		Sig. (2-tailed)	.018	.000	.720	.	.000	.055
		N	360	360	360	360	360	360
	Hardness mg/l	Correlation Coefficient	.561**	.910**	.788**	-.200**	1.000	.114*
		Sig. (2-tailed)	.000	.000	.000	.000	.	.031
		N	360	360	360	360	360	360
	Total Goiter Rate	Correlation Coefficient	.428**	.055	.000	.101	.114*	1.000
		Sig. (2-tailed)	.000	.297	1.000	.055	.031	.
		N	360	360	360	360	360	360

**. Correlation is significant at the 0.01 level (2-tailed).

*. Correlation is significant at the 0.05 level (2-tailed).

Table 4.6: Correlation of TGR with thyroid parameters and water contents

			T4 nm/l	T3 nm/l	TSH mu/l	Tg ng/ml	UI mcg/dl	USCN mg/l	CL mg/l	Ca mg/l	Mg mg/l	F mg/l	Hards mg/l	TGR
Spearmen	T4nm/l	Co.Coeff	1.000	.293 **	-.149 **	.002	-.173 **	.174 **	.000	-.149 **	-.179 **	-.008	-.137 **	.082
		Sig.(2-tailed)	.	.000	.004	.963	.001	.001	.998	.005	.001	.882	.009	.122
		N	360	360	360	360	360	360	360	360	360	360	360	360
	T3 nm/l	Co.Coeff	.293 **	1.000	.003	.014	.019	.128	-.256 **	-.211 **	-.168 **	.081	-.234 **	-.331 **
		Sig.(2-tailed)	.000	.	.958	.789	.720	.015	.000	.000	.001	.124	.000	.000
		N	360	360	360	360	360	360	360	360	360	360	360	360
	TSH mu/l	Co.Coeff	-.149 **	.003	1.000	.003	.175 **	-.069	.063	.178 **	.245 **	.069	.198 **	-.064
		Sig.(2-tailed)	.004	.958	.	.957	.001	.192	.234	.001	.000	.193	.000	.222
		N	360	360	360	360	360	360	360	360	360	360	360	360
	Tg ng/ml	Co.Coeff	.002	.014	.003	1.000	-.047	-.147 **	.147 **	-.058	.060	-.131	.000	-.073
		Sig.(2-tailed)	.963	.789	.957	.	.373	.005	.005	.275	.257	.013	.993	.165
		N	360	360	360	360	360	360	360	360	360	360	360	360
	UI mcg/dl	Co.Coeff	-.173 **	.019	.175 **	-.047	1.000	.081	.138 **	.191 **	.266 **	.269 **	.168 **	-.078
		Sig.(2-tailed)	.001	.720	.001	.373	.	.124	.009	.000	.000	.000	.001	.139
		N	360	360	360	360	360	360	360	360	360	360	360	360
	USCN mg/l	Co.Coeff	.174 **	.128	-.069	-.147 **	.081	1.000	-.015	-.046	-.079	.041	-.081	.071
		Sig.(2-tailed)	.001	.015	.192	.005	.124	.	.782	.383	.136	.441	.125	.179
		N	360	360	360	360	360	360	360	360	360	360	360	360
	CL mg/l	Co.Coeff	.000	-.256 **	.063	.147 **	.138 **	-.015	1.000	.434 **	.502 **	-.124	.561 **	.428 **
		Sig.(2-tailed)	.998	.000	.234	.005	.009	.782	.	.000	.000	.018	.000	.000
		N	360	360	360	360	360	360	360	360	360	360	360	360
	Ca mg/l	Co.Coeff	-.149 **	-.211 **	.178 **	-.058	.191 **	-.046	.434 **	1.000	.630 **	-.296 **	.910 **	.055
		Sig.(2-tailed)	.005	.000	.001	.275	.000	.383	.000	.	.000	.000	.000	.297
		N	360	360	360	360	360	360	360	360	360	360	360	360
	Mg mg/l	Co.Coeff	-.179 **	-.168 **	.245 **	.060	.266 **	-.079	.502 **	.630 **	1.000	-.019	.788 **	.000
		Sig.(2-tailed)	.001	.001	.000	.257	.000	.136	.000	.000	.	.720	.000	1.000
		N	360	360	360	360	360	360	360	360	360	360	360	360
	F mg/l	Co.Coeff	-.008	.081	.069	-.131	.269 **	.041	-.124	-.296 **	-.019	1.000	-.200 **	.101
		Sig.(2-tailed)	.882	.124	.193	.013	.000	.441	.018	.000	.720	.	.000	.055
		N	360	360	360	360	360	360	360	360	360	360	360	360
	Hardness mg/l	Co.Coeff	-.137 **	-.234 **	.198 **	.000	.168 **	-.081	.561 **	.910 **	.788 **	-.200 **	1.000	.114
		Sig.(2-tailed)	.009	.000	.000	.993	.001	.125	.000	.000	.000	.000	.	.031
		N	360	360	360	360	360	360	360	360	360	360	360	360
	Total Goitre Rate	Co.Coeff	.082	-.331 **	-.064	-.073	-.078	.071	.428 **	.055	.000	.101	.114	1.000
		Sig.(2-tailed)	.122	.000	.222	.165	.139	.179	.000	.297	1.000	.055	.031	.
		N	360	360	360	360	360	360	360	360	360	360	360	360

** Correlation is significant at the 0.01 level (2-tailed). Correlation is significant at the 0.05 level (2-tailed).

Fig. 4.2: UIE in schoolchildren from selected Sudanese cities

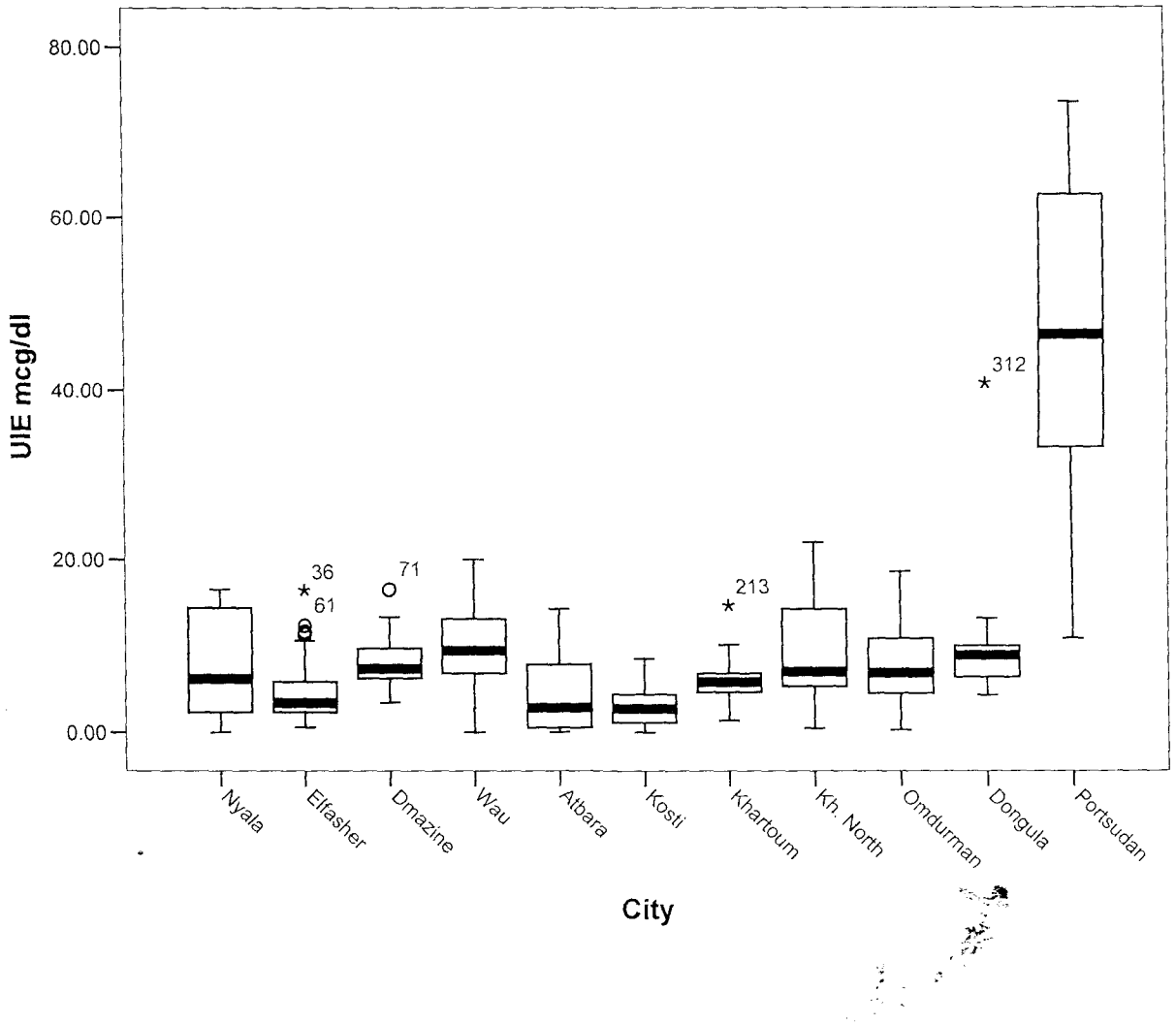


Fig. 4.3: UIE status in Sudanese schoolchildren according to the WHO/ UNICEF/ICCIDD criteria

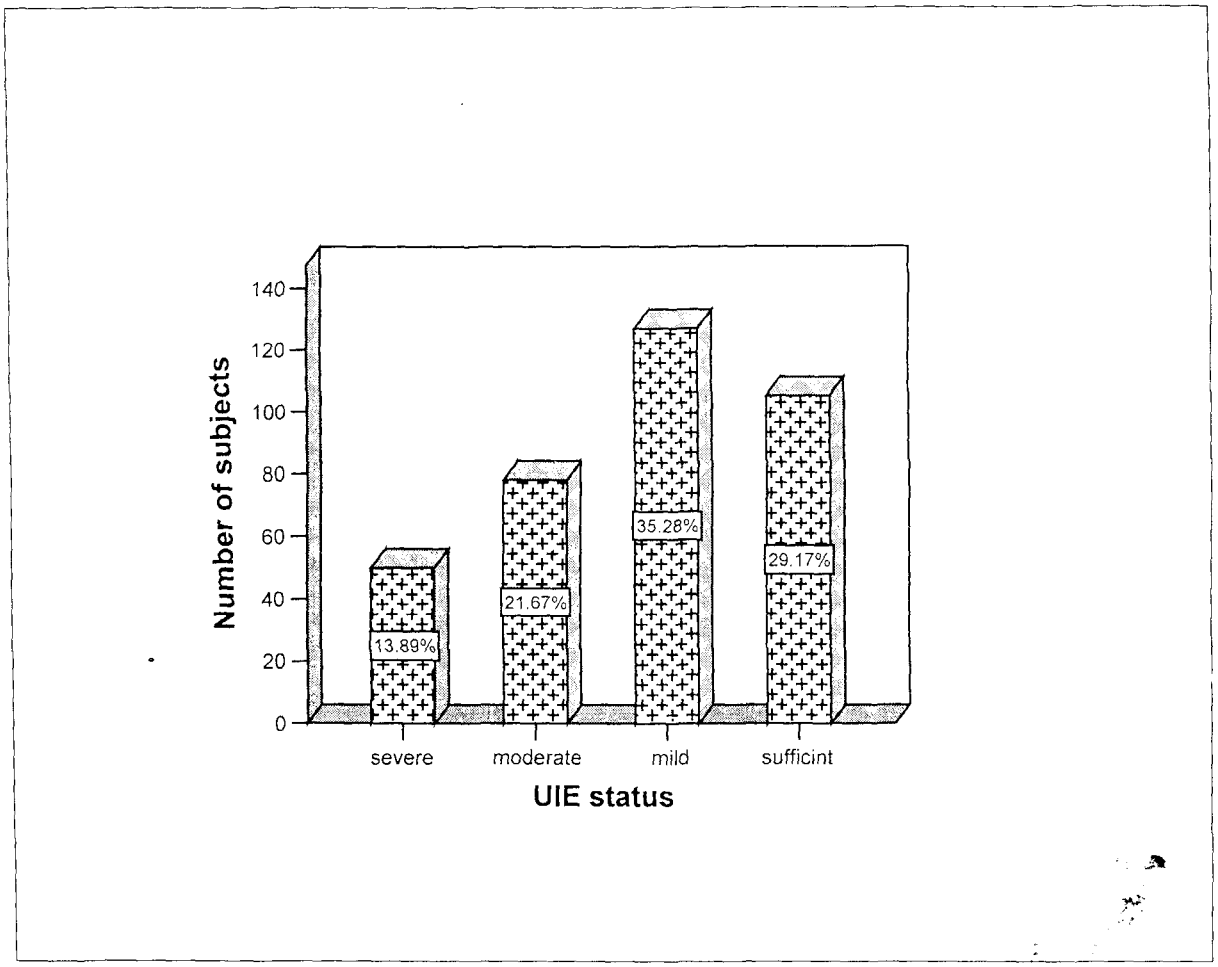


Fig. 4.4: USCN in Schoolchildren from selected Sudanese cities

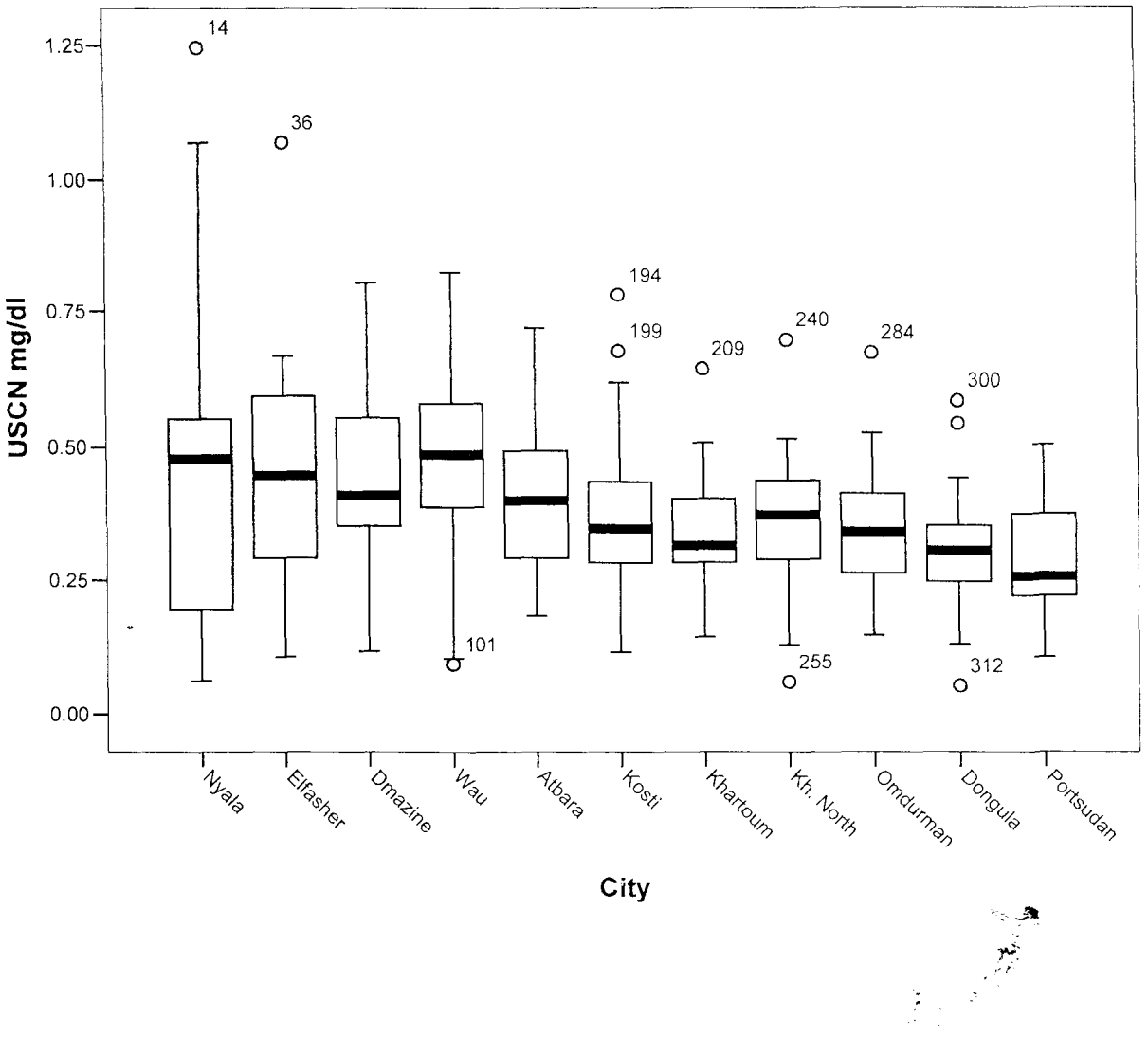


Fig. 4.5: USCN status in Sudanese schoolchildren according to the WHO/ UNICEF/ICCIDD criteria

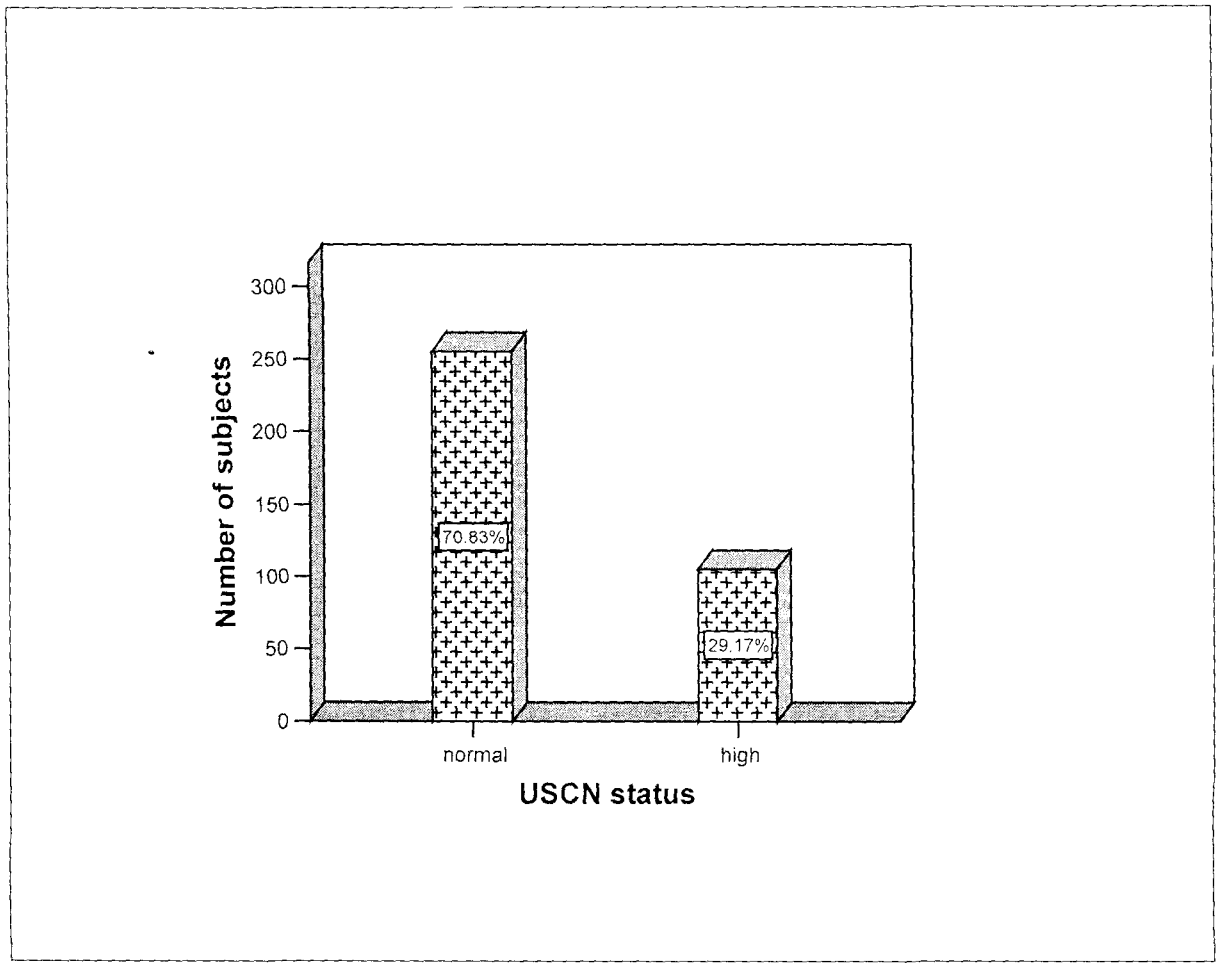


Fig. 4.6: Serum T_4 level in schoolchildren from selected Sudanese cities

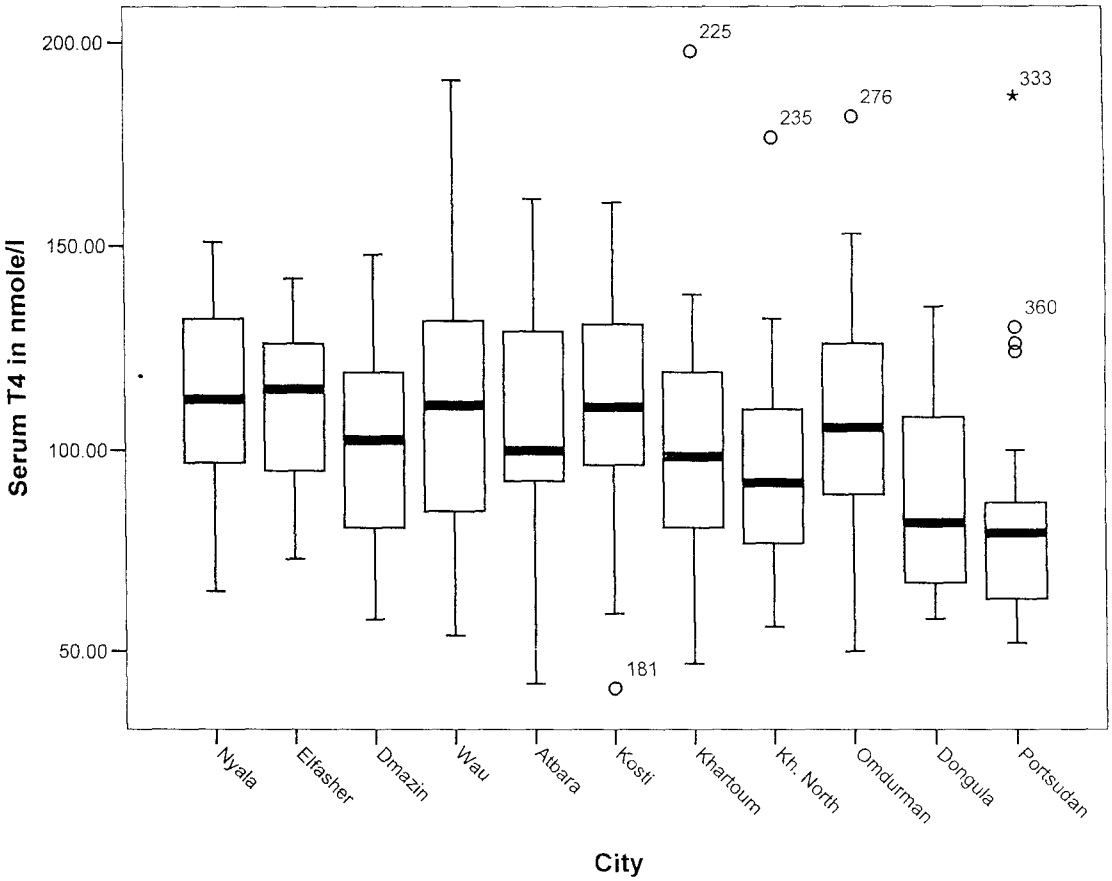


Fig. 4.7: Serum T_4 status in Sudanese schoolchildren according to the SAEC normal range

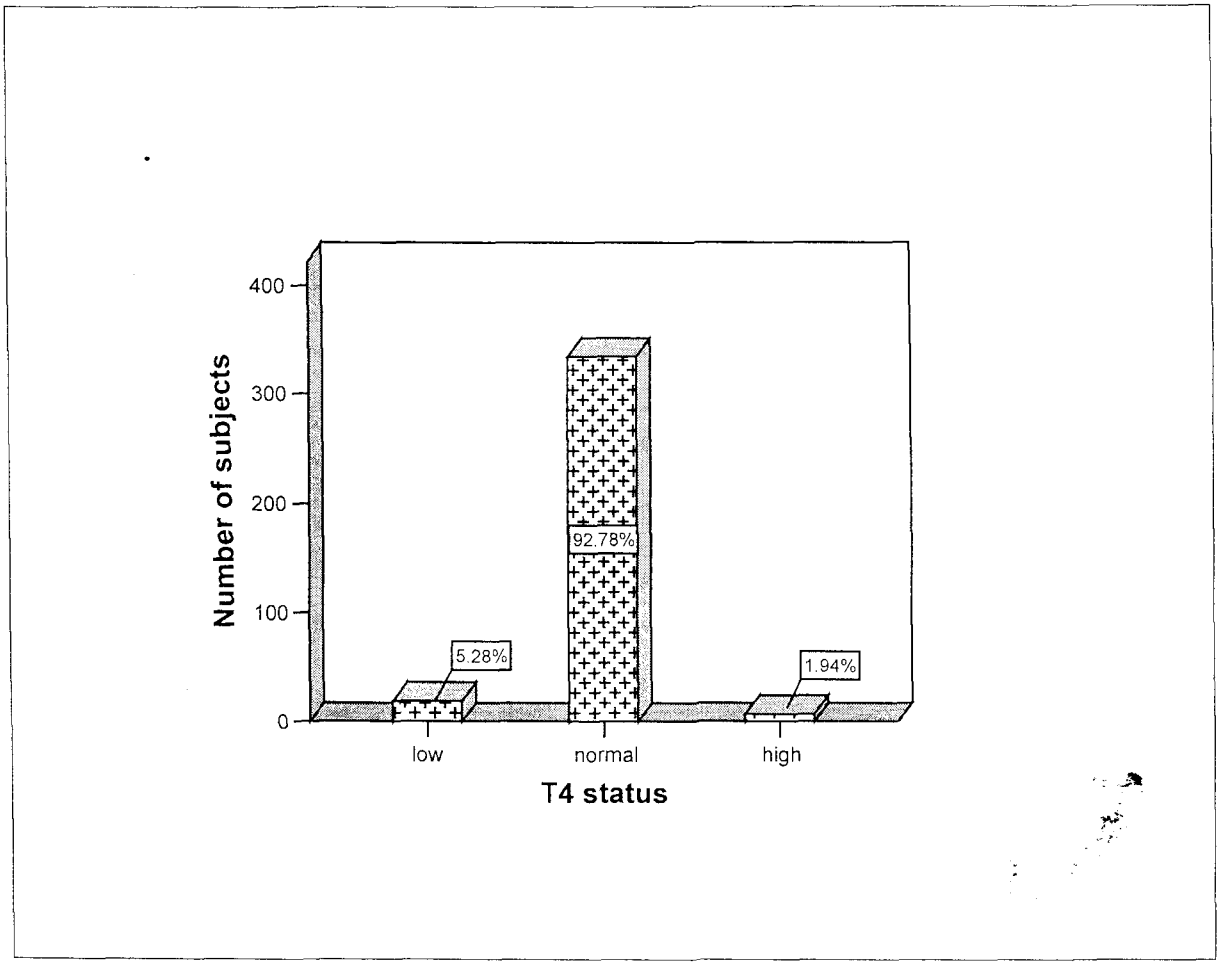


Fig. 4.8: Serum T_3 level in schoolchildren from selected Sudanese cities

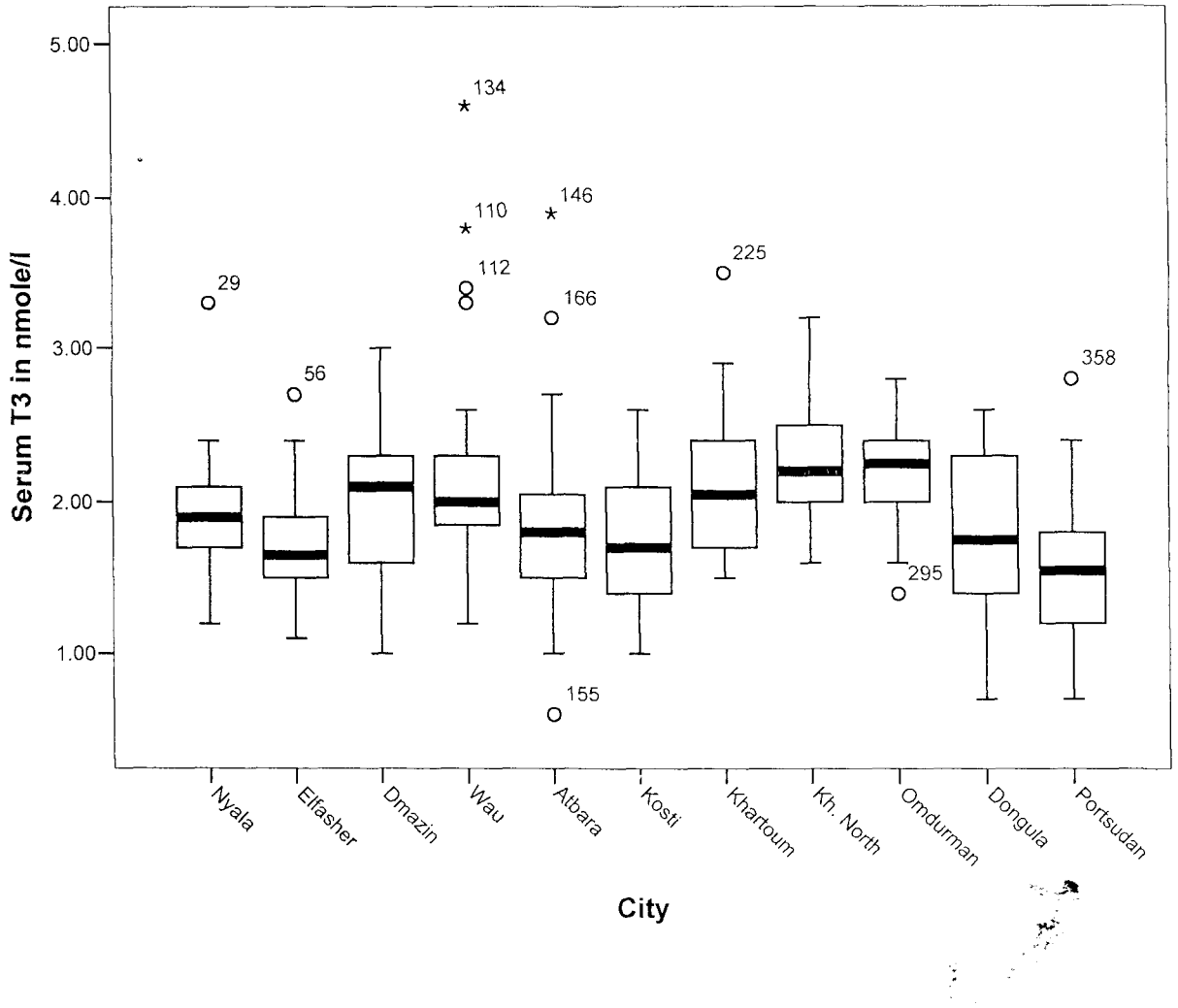


Fig. 4.9: Serum T_3 status in Sudanese schoolchildren according to the SAEC normal range

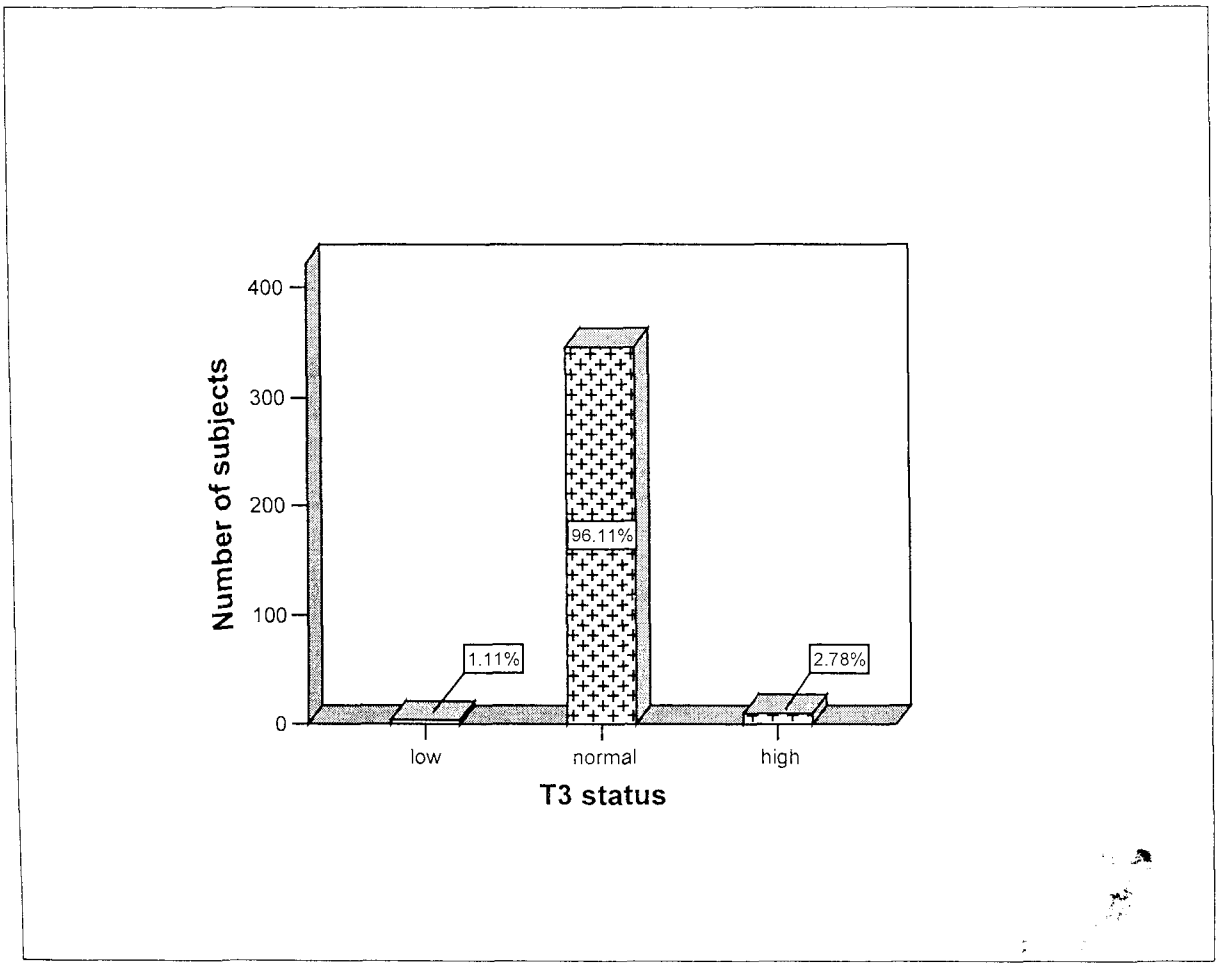


Fig. 4.10: Serum TSH level in schoolchildren from selected Sudanese cities

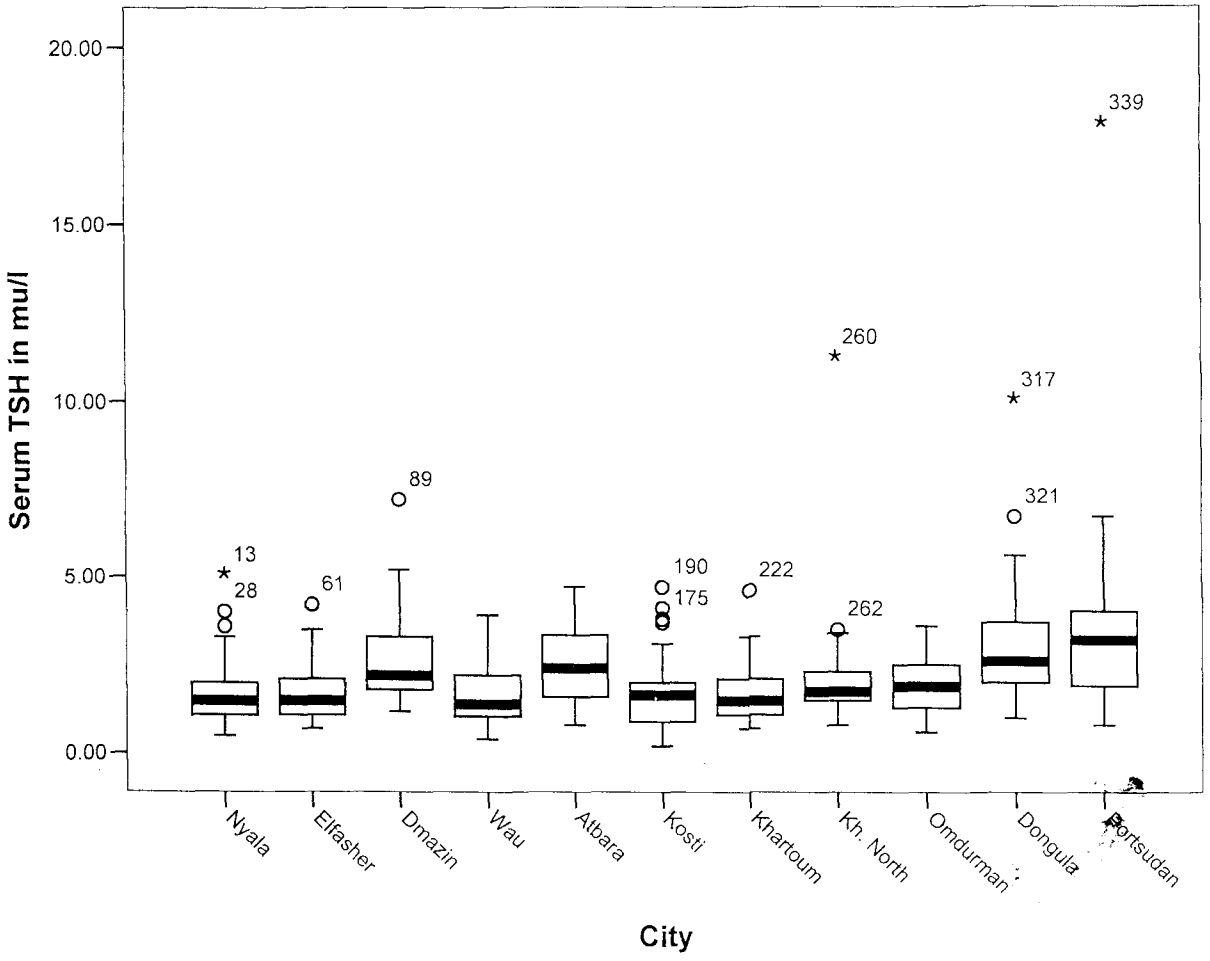


Fig. 4.11: Serum TSH status in Sudanese schoolchildren according to the SAEC normal range

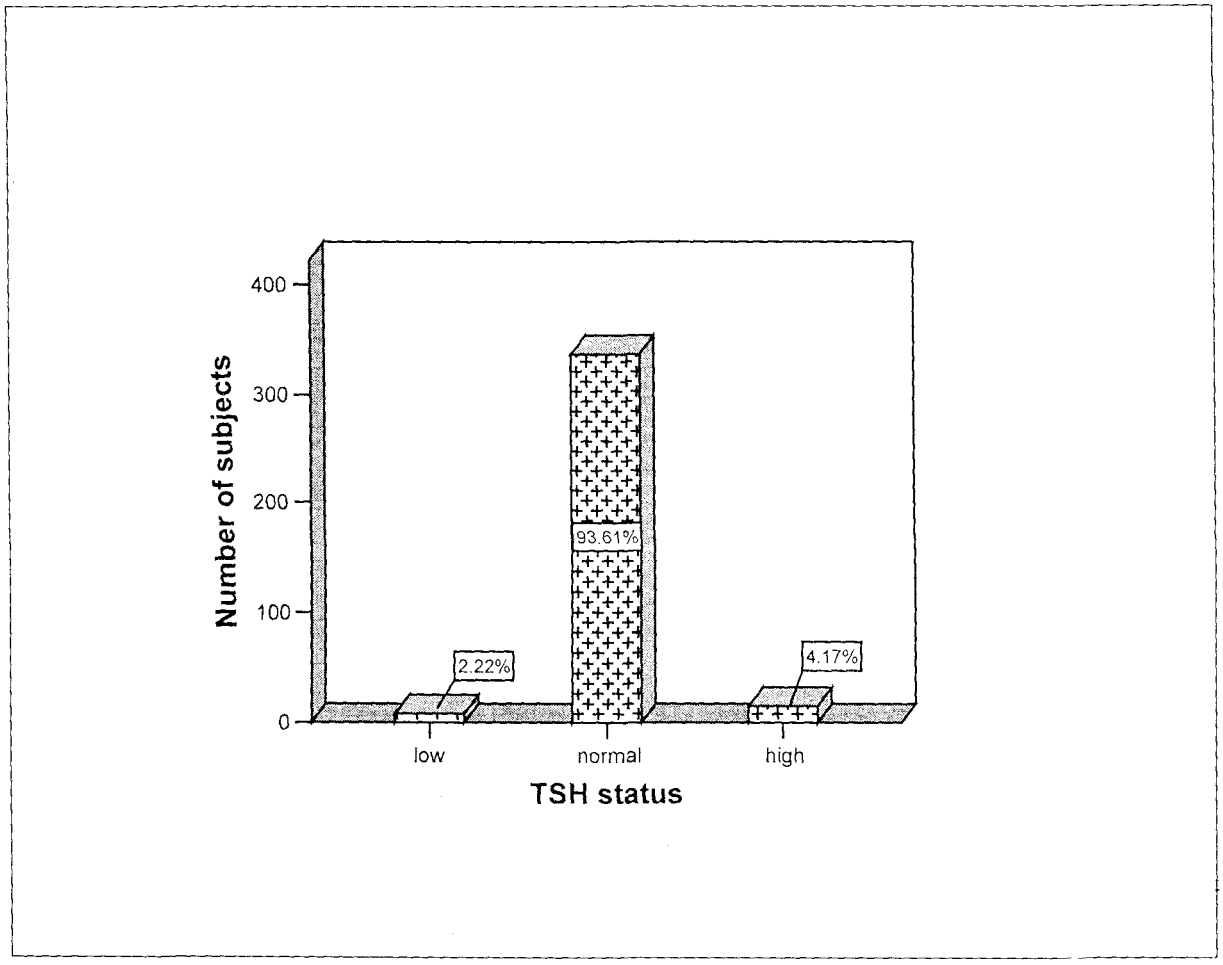


Fig. 4.12: Serum Tg level in schoolchildren from selected Sudanese cities

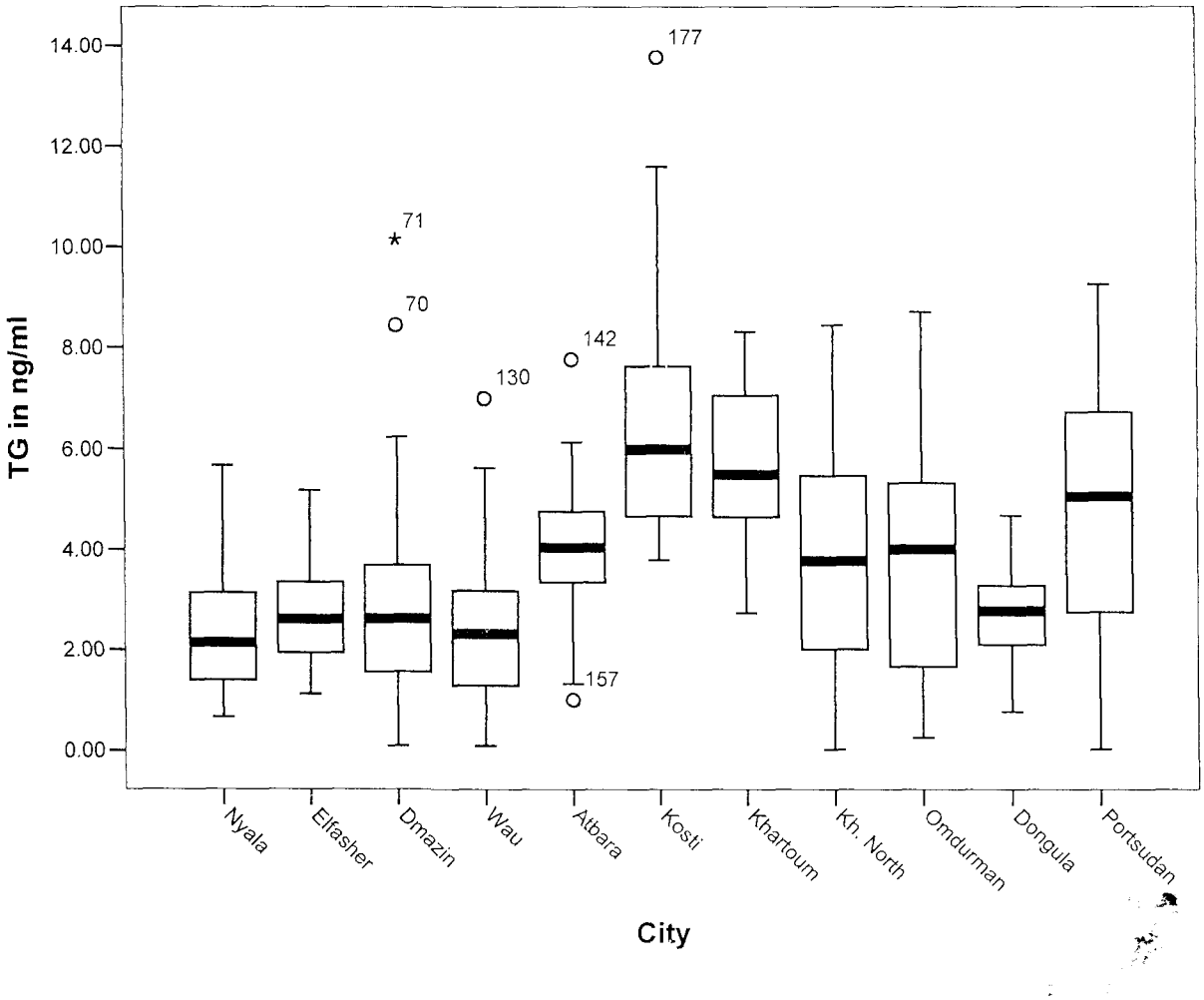


Fig. 4.13: Serum Tg status in Sudanese schoolchildren *in relation to UIE levels* according to the WHO criteria

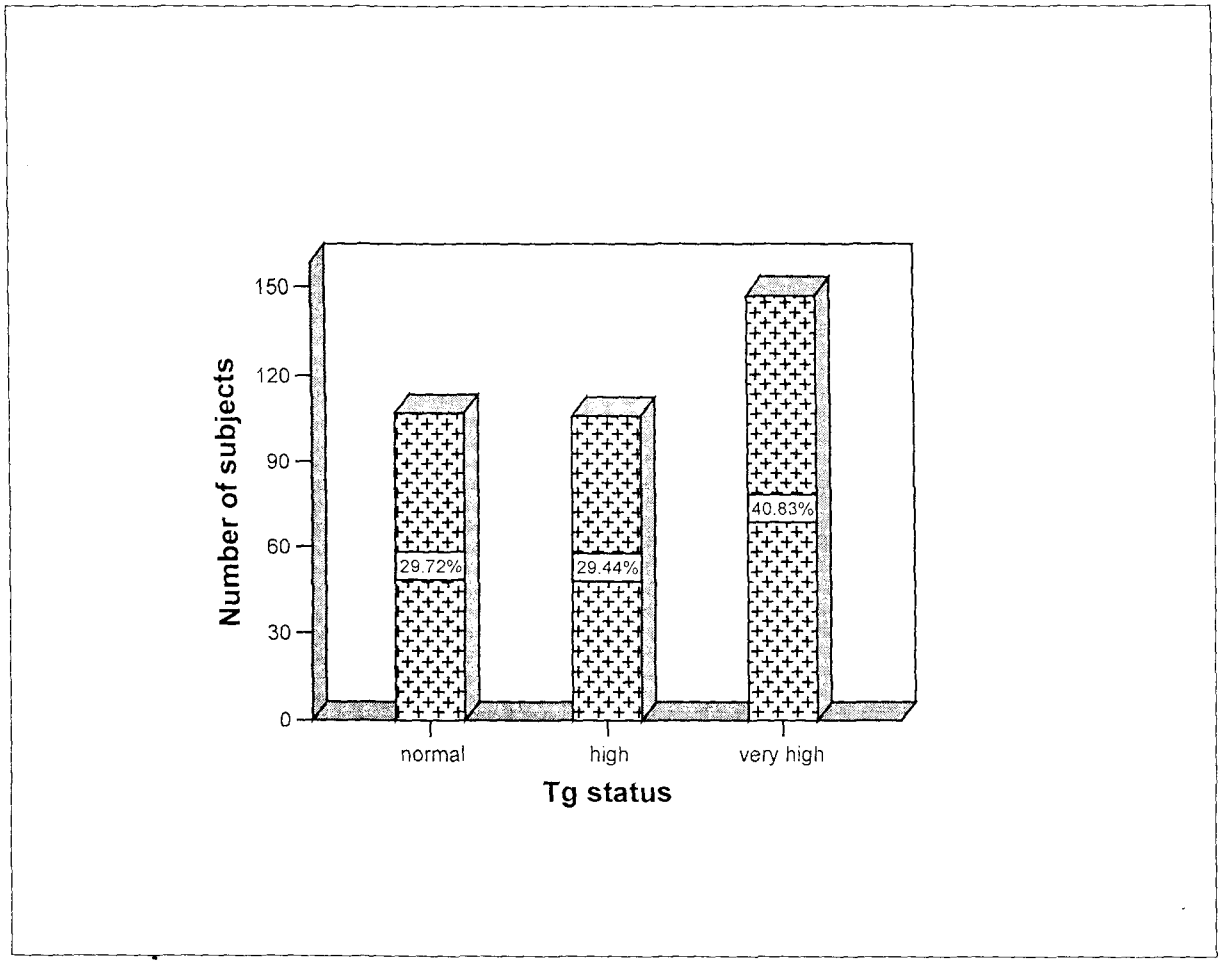


Fig. 4.14: Mean chloride concentration in drinking water collected from schools in selected Sudanese cities

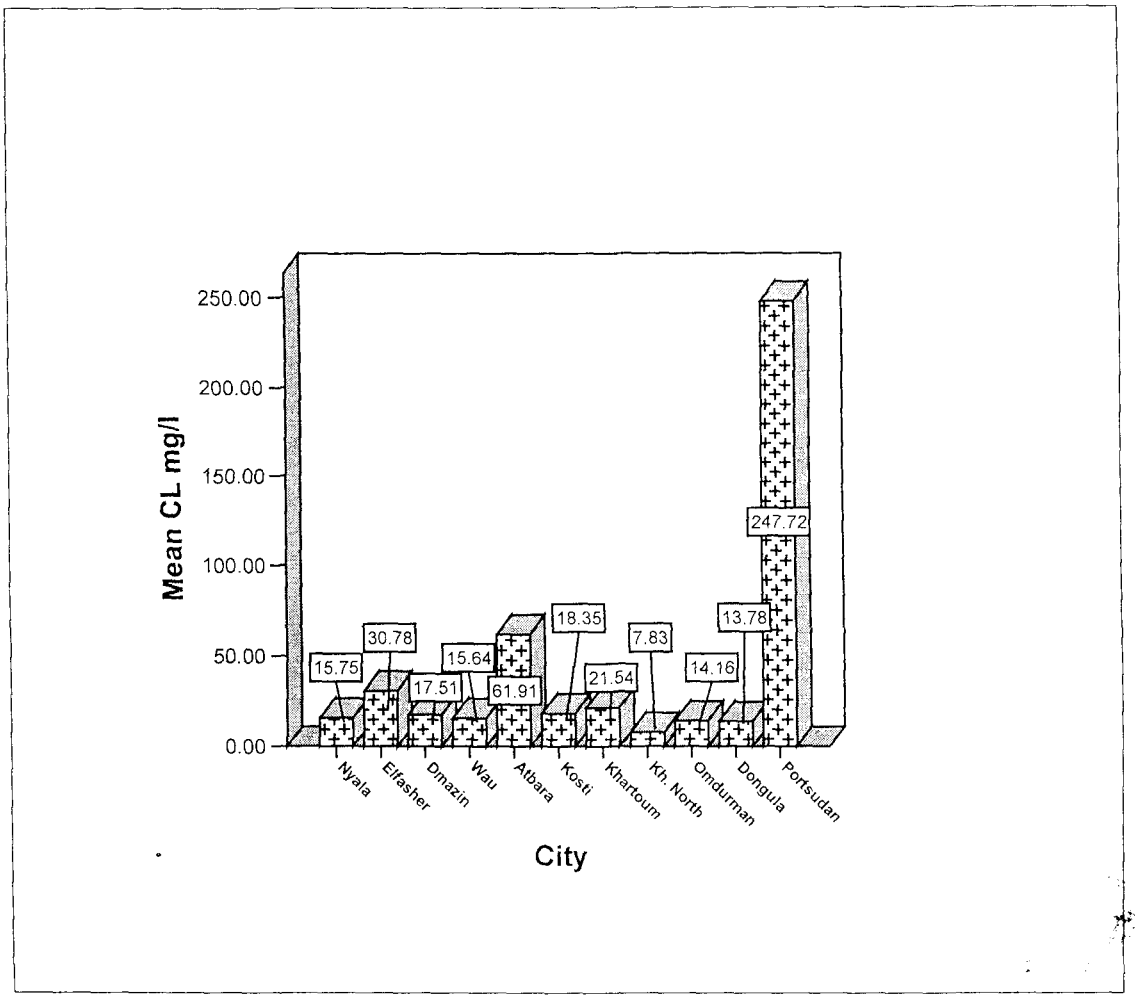


Fig. 4.15: Mean calcium concentration in drinking water collected from schools in selected Sudanese cities

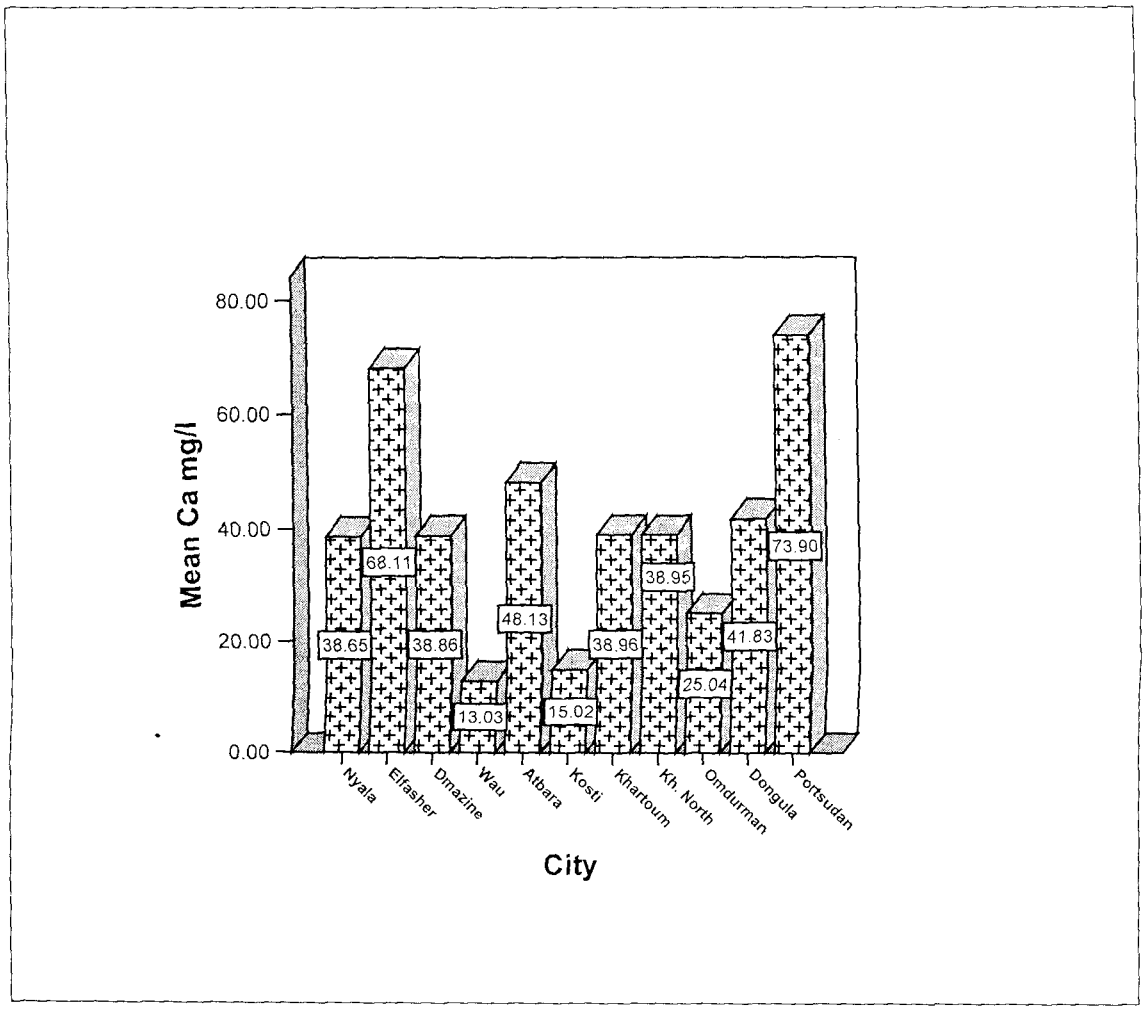


Fig. 4.16: Mean magnesium concentration in drinking water collected from schools in selected Sudanese cities

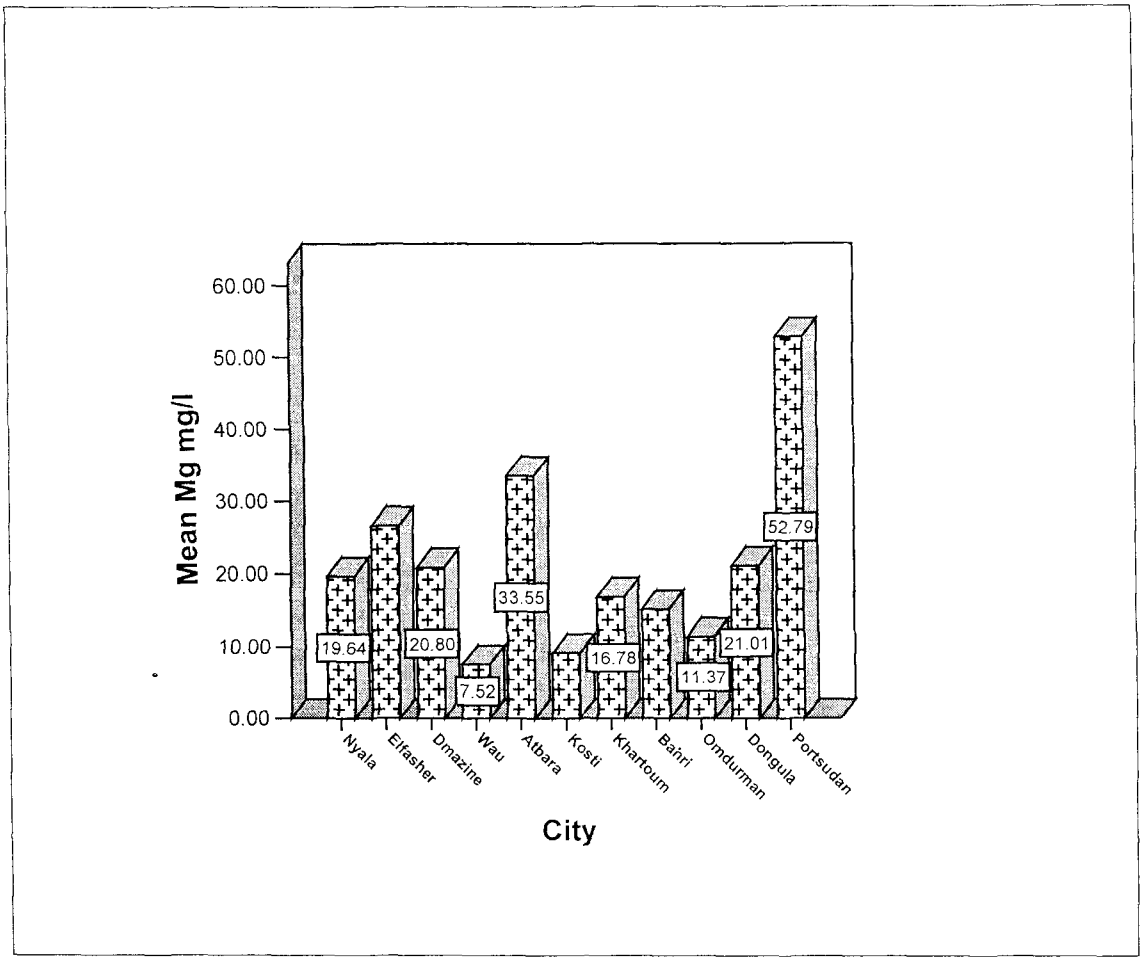


Fig. 4.17: Mean Total hardness ($CaCo_3$) concentration in drinking water collected from schools in selected Sudanese cities

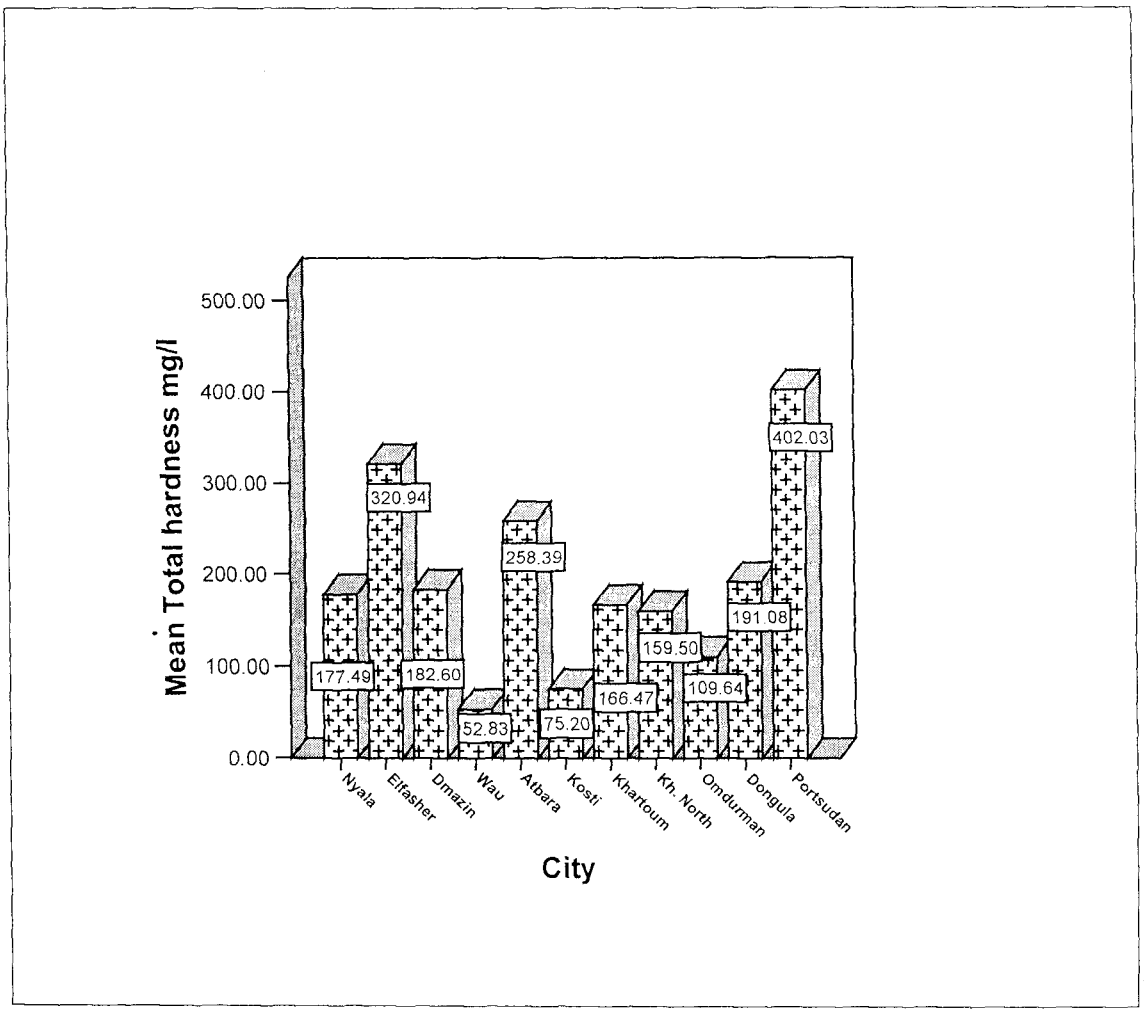
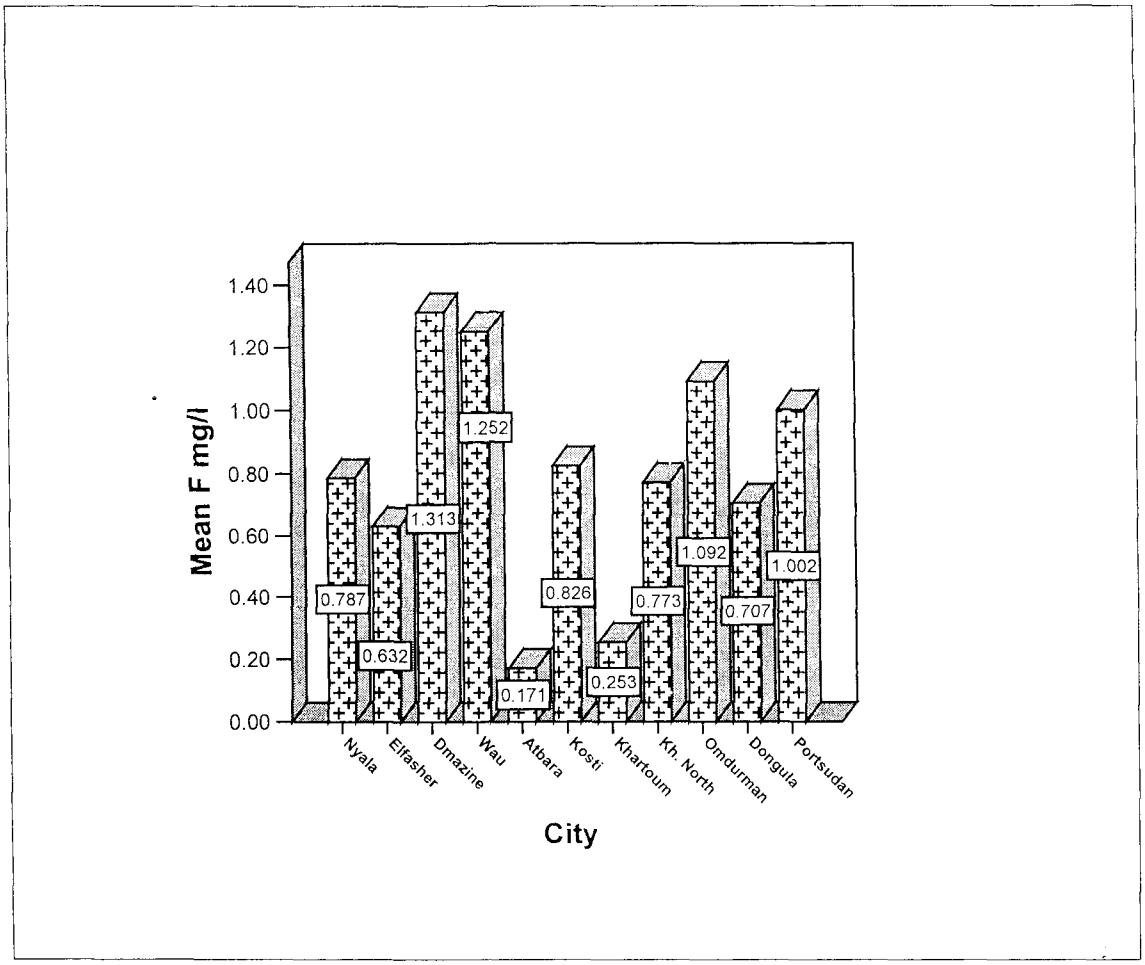


Fig. 4.18: Mean fluoride concentration in drinking water collected from schools in selected Sudanese cities



CHAPTER FIVE

DISCUSSION

The WHO/UNICEF/ICCIDD (WHO *et al.,* 1994) recommended that, the total goiter rate (TGR) and the level of urinary iodine excretion (UIE) in schoolchildren (6-12 years) are the most accepted markers for the evaluation of the severity of IDD in a region. The IDD is considered as a public health problem in a region when the prevalence of goiter in schoolchildren is 5% or more. Goiter prevalence of 5.0 - 19.9% is considered as mild, 20.0 - 29.9% as moderate and a prevalence rate of above 30% is considered as a severe public health problem.

Accordingly, the result of this study showed that goiter is prevalent at endemic level in all study areas. The total goiter rate obtained was 40.62% in the studied areas (Table 4.1) indicating that IDD is a severe public health problem in the country as a whole. Although, there is a time lag between this study and previous ones (Kambal *et al.,* 1967; Abdel Wahab *et al.,* 1984; Eltom, 1984; Eltom *et al.,* 1985; Abdel Monim *et al.,* 1993; Elnagar *et al.,* 1995; FMOH 1997; Elnour *et al.,* 2000), our results agreed with the majority of these studies that goitre is endemic in the all studied areas in Sudan. In agreement with the findings obtained by Elnour in the southeast Sudan, that TGR was 22.3%, while our result was 26.9% in the Dmazine "the biggest city in that part of the country". The present results showed that TGR in Khartoum, Khartoum North and Omdurman "the major three towns of Khartoum state" was 14.9%, 17.15 and 12.22%, respectively. The TGR reported in the previous studies for Khartoum was 17% (Eltom, 1984), 5% (FMOH, 1997) and 13.9% in Omdurman (Abdel Monim *et al.,* 1993). The present results showed that TGR was 77.67% in Kosti, 64.09% in Nyala and 66.01% in Elfasher. The results obtained in this study were comparable with those reported by Eltom (Eltom, 1984) that the TGR was 74.8% in Kosti town, 86.7 in Nyala city and 87.6% in the rural areas of Elfasher city. Ten years later Elnager (1995) found that TGR decreased to 79.6% in the rural areas of Darfur (Elnagar *et al.,* 1995). A similar trend was observed in the current study. The reason could be attributed to

the increasing awareness of the people in the Darfur region to the consequences of the IDD and their response to the efforts carried out by the local health authorities and the international organizations (UNICEF and WHO). In contrast to the improvement of thyroid status in Darfur region, the TGR in Kosti was getting worse compared with the finding obtained by Eltom in 1984. The data reported by Federal Ministry of Health (FMOH, 1997) in which the TGR was 28% in Darfur, 5% in Khartoum, and 8% in the eastern Sudan. The TGR reported by the FMOH were lower than the TGR reported in the previous studies. Furthermore, the TGR reported by the FMOH did not match the UIE of the people in these cities. In a very recent study (Hussien *et al.*, 2007), TGR in schoolchildren from Port Sudan city was found to be 17% while the TGR of schoolchildren in the same city obtained in this study was 50.15%. Although, both studies were conducted in the same period of the second half of 2006, still, they showed controversy. The overall TGR in this study population was 40.62%, while in the FMOH (1997) study the population was 40922 and the TGR was 22%, although there was a remarkable variation in the TGR between the two studies, the TGR is still reflecting endemicity of goitre in both studies. The population of this study was the schoolchildren of the urban areas of the selected cities who may have greater chances for food varieties and better iodine nutritional supply than the children in the rural areas.

The WHO/UNICEF/ICCIDD (WHO *et al.*, 2001) reported that, in a given region more than 50% of the schoolchildren should have urinary iodine excretion (UIE) level of 10 µg/dl or more, and not more than 20% of samples should have UIE level of less than 5 µg/dl. The present findings of the urinary iodine excretion level indicated that the overall studied population except Port Sudan had some degree of biochemical iodine deficiency. The median level of UIE for each city is shown in Table (4.3). The overall median UIE in this study was 6.55 µg/dl and the range was 0.02-73.6 µg/dl. The median UIE in 71.43% of the whole population was lower than the WHO cut off point (10µg/dl). Out of this 21.57% excreted less than

lower than the WHO cut off point (10µg/dl). Out of this 21.57% excreted less than 5 µg/dl, and 14.85% had median UIE lower than 2 µg/dl. The country as a whole will be classified according to the present findings as moderately severe iodine deficient.

Port Sudan has exceptional high median UIE (46.0 µg/dl) with a range of 10.9-73.6 µg/dl. The UIE level in this city indicated sufficient iodine intake in the whole pupils included in this study since they all excreted UI level above the WHO cut-off point. In agreement with the results obtained by Eltom (1984) that the mean UIE in subjects from Port Sudan was 190.0 ± 85.2 µg /g creatinine which was about 2.5 times as in subjects from Khartoum (83.6 ± 41.9 µg/g creatinine, and as 3 times as in subjects from the Darfur region (65.0 ± 65.4 µg/g) creatinine. Similar results were recently obtained by Hussien (Hussien *et al.*, 2007) that the median UIE in schoolchildren from Port Sudan was 55.52 µg /dl. This could be attributed to the fact that most people in this area consume seafood rich in iodine. A second source of iodine intake could be due to inhalation of iodine that evaporates from the sea and enters the atmosphere, it can become attached to dust particles or be precipitated in rainfall and enriches the soil (Vought, 1972). Iodide ions can be oxidized by sun light to volatile elemental iodine (Miyak *et al.*, 1963). It has been calculated that about 14-20 µg of iodine can be added to the daily iodine intake, since "standard man" breathes 20 m^3 of air per day (Koutras *et al.*, 1980).

This could explain the adequate iodine intake in the population studied. In contrary to the previous studies revealing that, coastal regions are associated with low prevalence of goiter due to iodine sufficiency worldwide (Kabil 1998). Surprisingly, the goitre rate in Port Sudan city was 50.15%. However, similar observations were obtained in coastal areas in India such as Kerala, Panaji, Pondicherry, and Bombay, reported high goiter prevalence rate of 16.6% to 43% (Kapil *et al.*, 1996; Dodd, 1993; Sarkar *et al.*, 2007). The authors attributed this high prevalence of goiter in these iodine sufficient areas to the consumption of

cyanogenic food. Lack of relation between the UIE and the TGR in Port Sudan could be attributed to interference of potent goitrogenic factors in the food and/or drinking water.

The result of this study indicated that the iodine deficiency is the major etiological factor to the high prevalence of goiter in the populations studied (except Port Sudan). The same findings were reported in previous studies in Sudan (Kambal, 1967; Eltom, 1984; Eltom *et al.*, 1985; Abdel Monim, 1993; Elnagar *et al.*, 1995; Abdel Wahab *et al.*, 1984 and FMOH, 1997). The lowest UIE (2.70 µg/dl) was detected in Kosti city in which TGR was the highest (77.67%) compared with the other Sudanese cities. Goiter prevalence is indicative of past iodine status while UIE levels indicate the present iodine intake of the population. In the present study, both of the indicators were positive, indicating the high endemicity of goitre and severity of the IDD consequences.

The city of Atbara was not included in the previous goitre surveys. The distinguished location of Atbara between the capital Khartoum and Port Sudan in the Eastern side as well as between Khartoum and the Northern states, one would expect diversification in food resources, but contrary to this more than 85% of the pupils in this city had low iodine intake. The median UIE in the schoolchildren in Atbara was 2.80 µg/dl, only 5 pupils out of 35 had sufficient UIE. The results of this study may also support the previous findings that goitre is still spreading in new areas of the country. TGR of these populations was not correlated with the severity of iodine deficiency across the cities studded. This observation could explain the presence of goitrogenic and/or anti-thyroidal substances, which may interfere with the iodine uptake by the thyroid gland and increase its excretion in the urine. Or may aggravate the effects of iodine deficiency and increase thyroid anomalies and the endemicity of goiter in these areas of the country. The action of these goitrogenic factors may not be antagonized by the presence of highly sufficient iodine intake as in Port Sudan city.

The thyroid hormones results showed that the mean serum TSH, T_4 and T_3 concentrations were within the normal reference range. A strong correlation was observed between mean serum T_4 and T_3 and they both negatively correlated with the mean serum TSH levels. This result may indicate that the thyroid gland can adapt itself to iodine deficiency and produce sufficient hormones under the normal stimulatory action of pituitary gland. It can maintain the normal thyroid-pituitary feedback pattern. It was reported that (Delange, 2000b) when iodine intake is abnormally low, adequate secretion of thyroid hormones may still be achieved by marked modifications of thyroid activity. This normal secretion of thyroid gland can be obtained under the effects of iodine deficiency as well as the presence of different goitrogenic factors. The same observations were obtained in a similar goiter epidemiological study in Benin (Briel *et al.*, 2001) in which normal values of serum TSH and FT4 were obtained in iodine deficient schoolchildren. It was concluded that, serum concentrations of TSH and of FT_4 could not be regarded as appropriate indicators for detecting moderate-to severe iodine deficiency in children at school age. They also reported that the normal range for the thyroid hormones is too wide to predict IDD status. The lack of relationship between serum TSH level and goitre size may be due to differences in the duration of elevation of TSH , or , to individual variation of thyroidal responsiveness to TSH and / or to participation of other factors (Chopra *et al.*, 1975) such as selenium deficiency in some African countries (Zimmermann, 2004). It is postulated that, there may be cellular defects of the follicles leading to a sequestration of iodine compartments with slow iodine turn over. Stimulation by TSH would increase the inefficiency of these intrathyroidal pathways.

Thyroid hormones results obtained in this study may add more confirmations to what was reported by the WHO (WHO *et al.*, 2001) that, determination of serum concentrations of the thyroid hormones (T_4 and $T_{3)}$ usually impractical for monitoring iodine nutrition. There is a large overlap to make these tests not advisable for ordinary epidemiological purposes. The difference between iodine-

deficient and iodine-sufficient population groups is neither great nor consistent, much overlap occurs between individual TSH values. Therefore, the blood TSH concentration in school-age children and adults is also not a practical marker for iodine deficiency, and its routine use in school-based surveys is not recommended (WHO *et al.*, 2001).

The results of this study showed that, the UIE was significantly correlated with the serum TSH ($P<0.001$) and negatively with serum T_4 ($P<0.001$). This could be explained by the presence of goitrogenic factors, which lead to inhibition of iodine uptake by the thyroid and increased iodine clearance in the urine. The unavailability of the iodine for the thyroid gland resulted in a decrease of thyroid production of thyroxin and consequent increase TSH in production by the pituitary gland.

Measurement of serum thyroglobulin (Tg) recommended by the WHO in 1994 as a more sensitive indicator for thyroid cell mass and a good monitor for iodine status than the other three indicators (UIE, TGR, and serum TSH) (WHO *et al.*, 1994). The WHO also proposed that a median Tg concentration of less than 10 µg/liter in a population indicated iodine sufficiency (WHO *et al.*, 1994). However, data confirming Tg cutoff value were limited.

Recent studies (Dunn & Dunn, 2000; Briel *et al.*, 2001; Zimmermann *et al.*, 2006) reported that serum Tg is elevated in iodine-deficient areas due to TSH hyperstimulation and thyroid hyperplasia. There were technical problems in measurements of Tg including large interassay variability and lack of reference data for Tg in healthy, iodine-sufficient school-age children (Zimmermann *et al.*, 2006).

The normal mean values for serum Tg reported by Vanderschueren-Lodeweyckx (1996) in children aged 1–10 year was 2– 65 µg /liter and 2–36 µg /liter for children of 11–20 year old. It has been calculated by Zimmerman and his group (2006) that the Tg reference interval for iodine sufficient, Tg-Ab-negative, euthyroid school-age children was 4–40 µg /liter. The reference value obtained

using Dried Blood Spot (DBS) method with European Reference standards (CRM-457-standards).

In another study in 83 iodine deficient Moroccan schoolchildren Zimmermann reported that median Tg was 49 µg/liter at the baseline. Tg fell rapidly after introduction of iodized salt to 13 and 8 µg/liter at 5 and 10 months (Zimmermann, 2003). It was also reported in the same study that Tg was negatively correlated with urinary iodine and positively correlated with thyroid volume and TSH.

In an international study (Zimmermann *et al.,* 2006) to develop a reference standard and to establish a new international reference range for Tg, the median Tg concentration was measured using DBS method in samples of 5-14 year old euthyroid children from different areas of long-term iodine sufficiency. The study included 698 children from Bahrain, Peru, South Africa, China, and Switzerland. The median Tg was 19.3, 11.6, 18.4, 13.3 and 11.2 µg/liter, respectively. The overall median was 14.5 µg/liter. The median serum Tg in this study was 34.1 µg/l with the range of 0.3 – 137.7 µg/l.

One of the objectives of this study is to introduce serum Tg level as an indicator for iodine nutritional status and IDD assessment. Unfortunately, the normal range or the cut-off point for serum thyroglobulin was not determined in our laboratory or in any other laboratory in Sudan. Although the highest median Tg concentration was detected in Kosti city where the UIE was the lowest (2.70 µg/dl), and the TGR was the highest (77.67%), there were no significant correlation seen between serum Tg and other thyroid related hormones or the urinary excretion of iodine and SCN levels.

The median serum Tg in this study was 34.1 µg/l in less severe iodine deficient Sudanese children was lower than that of 49 µg/l obtained by Zimmermann (2003) from severe iodine deficient Moroccan children and higher than the international median value (14.5 µg/liter) obtained in iodine sufficient countries (Zimmermann *et al.*, 2006). The median serum Tg in this study was also higher than the cut off point estimated by the kit producer (24.5 µg/l) and the cut off point (10 µg/l)

estimated by the WHO. It could be concluded that the Tg values in this study could be ranked as intermediate sufficient and severe iodine deficiency regions.

The urinary iodine excretion and the goiter rate in this study showed moderate severity of iodine deficiency comparable with the significant elevation of the serum Tg level. Thus, the median serum Tg concentration could be used as a significant indicator for monitoring iodine nutrition and IDD status. We could also take these values as base line data for the future analysis after distribution of iodized salt. It has been confirmed that Tg decreases rapidly with iodine repletion, which makes Tg to be a sensitive indicator of iodine repletion than the thyrotropin or thyroxin (Todd & Dunn, 1998; Zimmermann, 2003).

In this study, the overall mean urinary excretion of thiocyanate was 0.39 ± 0.17 mg/dl with median of 0.37 mg/dl and a range of 0.05-1.25 mg/dl. The median USCN excretion in subjects from Nyala, Elfasher, Wau and Dmazine were slightly higher than in the subjects from the rest of the country. Similar trends were reported in the previous studies (Osman & Fatah, 1981; Eltom, 1984, Gaitan *et al.*, 1989; Elnour *et al.,* 2000). The high concentrations of thiocyanate in the urine samples of the studied populations in these regions might be attributed to the consumption of millet and sorghum in Darfur and Dmazine regions. The highest median USCN was detected in the pupils from Wau city (0.49 mg/dl). Wau region was not studied before but high concentration of thiocyanate most probably attributed to consumption of cyanogenic plants such as cassava that is available in southern Sudan.

During the surveillance in this study, we have observed that the mean staple food is the bread made from imported flour followed by sorghum, at least amongst the populations of the urban areas of the selected cities. However, in Sudan there is a remarkable diversity in the types of food consumed according to the diversity in geographical and ethnical background. Some types of food containing cyanogenic plants rich in SCN compounds. Nevertheless, the food consumption depends on the availability of the locally produced plant especially for the poor people in the

small cities. Thus, food types are varied from time to time according to the season of production and from region to another according to the availability of transportation. In the western and southern states, the villages are more isolated and scattered in huge areas. The food resources are limited in the rainy seasons. This is because of the lack of paved roads. One of the aims of this study is to evaluate the urinary SCN status in different parts of the country and postulate its possible role in the etiology of goiter. There were no local or international clear cut-off point for thiocyanate concentration as goitrogenic factor in the urine samples. However, there were huge data allover the world referring to the role of thiocyanate at different levels in the etiology of goiter (Delange *et al.,* 1982; Laurberg *et al.,* 2002; Zimmermann, 2004). It has also been confirmed that thiocyanate has concentration dependent antithyroid properties (Brauer *et al.,* 2006).

Urinary thiocyanate values in the European communities was ranging between 40 µmol/l (0.23 mg/dl) (Lundquist *et al.,*. 1979) and 52 µmol/l (0.3 mg/dl (Lagasse *et al.,*. 1982). The mean urinary thiocyanate levels reported in different parts of India were 0.504 mg/dl (Marwaha *et al.,* 2003) and 0.708 mg/dl (Chandra *et al.,* 2006). It was reported that, urinary SCN excretion correlated positively with thyroid volume. The authors reported that supplementation of extra iodine failed to counteract the effect of SCN. In similar studies in different parts of India, in spite of adequate iodine intake, IDD remain a major health problem in many regions of India, the high TGR was due to consumption of cyanogenic plant foods available in India (Chandra *et al.,* 2004).

High urinary SCN concentrations were reported in different parts of Africa due to consumption of millet and cassava (Vanderpas *et al.,* 1984). It has been reported that (Thilly *et al.,* 1993) the USCN level in schoolchildren was 330 µmol/l (1.90 mg/dl) in Zaire and 170 µmol/l (0.98 mg/dl) in Malawi. It was reported that Millet consumption in Sudan and cassava in Zaire was found to aggravate the goiter

endemism in these places. In Nigeria thiocyanate levels in urine samples was 0.475 to 1.71 mg/dl (Eminedoki *et al.,* 1994).

The cut off point for urinary thiocyanate excretion reported by Peterson (2000) was 80 µmol/l (0.46 mg/dl), levels above 400 µmol/l (2.6 mg/dl) were considered highly elevated.

In Sudan, several studies on the role of SCN in the etiology of goiter took place. In 1984 Eltom reported that the additional contribution of thiocyanate as goitrogenic factors in rural Darfur induces thyroid abnormalities greater than that caused by the iodine deficiency alone in subjects from urban Darfur. The results of this study agreed with the results obtained by Eltom that thiocyanate was lower in the urine samples of subjects from Khartoum compared with those obtained in Darfur. But contrary to the result reported by Elnour (2000) in Blue Nile state (Southeast Sudan) that the mean values of USCN in pre-schoolchildren (1 to 6 years) was 259 µmol/l (1.49 mg/dl) this difference could be attributed to the age group used in each study. In comparison with the international findings concerning the level of SCN in the urine samples, the level of USCN obtained in this study was higher than in the European populations and lower than the levels reported in the subjects from India and some African communities. With reference to the cut-off point reported by Peterson (Peterson, 2000) for USCN which was 0.46 mg/dl, compared to the current findings, USCN elevated in 105 pupils out of 360 (29.17%) as is shown in Fig. (4.5). It could be shown that thiocyanate was a goitrogenic factor at least in the population of southern and western Sudan. However, the consumption of cyanogenic compounds was not evident in the studied populations from east (Port Sudan), center (Khartoum, Khartoum North and Omdurman) and Northern states (Atbara and Dongula) as detected from the urinary excretion of thiocyanate. It could also be pointed out that goitrogenic factors other than thiocyanate may be involved in the etiology of goiter in other regions of the country.

Similar observations were detected by previous studies (Kambal *et al.,* 1967; Eltom, 1984; Osman & Fatah, 1981; Abdel Wahab *et al.,* 1984; FMOH, 1997;

Elnour *et al.*, 2000). It was also reported that anemia, proteinenergy malnutrition and vitamin-A deficiency were health problems in some areas of the country (Elnour *et al.*, 2000). It has been confirmed that millet contained little if any thiocyanate (Gaitan *et al.*, 1989; Elnour *et al.*, 1998). However, it was confirmed that millet contains potent anti-thyroid flavinoids mainly C-glycosylflavones or C-GF (Gaitan *et al.*, 1995). Sorghum, the widely consumed staple food in Sudan, was assumed to be the major source of thiocyanate and flavonoids in the preschool subjects from Southeast Sudan (Elnour *et al.*, 2000). It has been reported that (Eltom, 1984) contaminated drinking water obtained from superficial wells in rural Darfur, and untreated White Nile river water in Kosti town may have some goitrogenic and antithyroidal effects in these areas.

In this study, the analysis of drinking water for chemical components indicated that the concentration of Ca, Mg, Cl, F and total hardness were within or slightly higher than the maximum permissible concentration reported by the WHO (WHO's drinking water standards 2006). There were still strong correlations between the concentration of these chemicals and the thyroid parameters. The results of this study showed that (Table 4.6) Cl content of the water was significantly proportional to the TGR ($P<0.000$), serum Tg ($P<0.005$), UIE ($P<0.01$), and negatively proportional to the serum T_3 concentration. Chloride was repeatedly been incriminated as a goitrogenic factor in the literature. Konova *et al.*, (1999) revealed that consumption of drinking water containing different concentrations of chlorine (0.3 to 1.8 mg/l) for different period were associated with a significant increase in follicular epithelial cell height of the thyroid gland in lambs. The results suggested antithyroidal effects of chlorine or chlorinated products formed when chlorine reacts with organic matter in the upper gastrointestinal tract. Similar findings were reported by (Bercz *et al.*, 1982; Orme *et al.*, 1985). In these studies, decreased serum thyroxin levels occur in monkeys and neonatal rats during subchronic exposure to chlorine dioxide in drinking water. Chloride was experimentally demonstrated as goitrogenic factor in mice

and monkeys (Bercz *et al.*, 1982). The present results showed also that, Ca content of water is strongly interfering with the thyroid activity, since Ca concentration was significantly correlated with serum TSH ($P<0.001$), UIE ($P<0.000$) and inversely correlated with serum T_3 ($P<0.000$) and T_4 ($P<0.005$). Ca was found to be goitrogenic in rats on a low iodine diet (Taylor, 1954).

The total hardness of water in this study was also significantly correlated with the other water contents (Table 4.6) as Ca ($P<0.000$), Mg ($P<0.000$) and Cl ($P<0.000$) and inversely correlated with F concentration ($P<0.000$). The goiter prevalence increased with increasing water hardness ($P<0.05$) and with fluoride concentration but the correlation was insignificant ($P=0.055$). The total hardness of water was significantly correlated with serum TSH ($P<0.000$), and inversely correlated with the concentration of serum T_4 ($P<0.01$) and serum T_3 ($P<0.000$). The findings of this study agreed with the results obtained by Day & Powell-Jackson (1972) who reported that the prevalence of goiter in 13 Himalayan villages was not attributed to differences in the low iodine intake. Instead, goiter prevalence was correlated closely with the fluoride content and the hardness of the water in each village. They also reported that effects of fluoride and water hardness seem to be independent. It was also reported that, increased dietary goitrogens SCN and hardness of water (although the total hardness did not exceed the upper limit reported by WHO) were associated with high goiter prevalence among the schoolchildren and adult inhabitants in an iodine deficient Plateau State of Nigeria (Das *et al.*, 1989). The present results also agreed with observation suggested by Fuge and Long (1989) that calcium and magnesium are considered additional goiter-inducing factors. The increased concentrations of these cations in the water lead to the presence of goiter in some areas without notable iodine deficiency in local environments. They also added that, in some areas, iodine deficiency might also be induced by fixation of iodine by calcium in the soils, yielding low concentrations in ground waters and decreased efficiency of uptake by plants. Similar findings were obtained by Gaitan in Colombia that there was a significant

relationship between the prevalence rate of goiter and the geological composition of water strata and watersheds (Gaitan, 1990). He observed that villages located in the lower stream of high organic rocks tend to have higher TGR compared with areas collecting drinking water from rivers going through low organic rocks. In contrary to the result reported by Malamos (1971) that the prevalence rate of goiter was negatively related to the calcium content of soil, and was not related to calcium content of the drinking water.

The high chemical contents of the drinking water could explain the high prevalence of goiter in Port Sudan city where the iodine intake is sufficient as evidenced by the elevated UIE. Furthermore, we have measured some chemical elements in the water available for the pupils at their schools during the working hours, but they may consume much quantities of water with different concentration of goitrogenic substances at their homes and the surroundings during the schools off hours. Some other chemicals such as nitrate and sulfate which are not included in this study may be present in higher concentrations and may pose more potent anti-thyroid activity effects. It has been reported (Gaitan, 1973) that shallow water table with high nitrate content due to human and animal excreta may have goitrogenic effects that contribute to the goiter endemicity.

We have observed during the course of this study that there were great variations in the quality of water available for pupils. Water samples had different taste, physical appearance (turbidity, colour, and some times smell), quality of the reservoirs, the cups and the way they provide water to the schools. There is a high risk pollution and bacterial contamination hence the water has been stored in an open sink in the ground and a very few dirty plastic containers available in some schools. In the larger cities, specially those adjacent to the rivers, schools were scattered in large areas where there were great variations in the water resources yield more variations in the water contents of chemicals and microbiological organisms. In contrary to the pupils in the small cities like Wau, Port Sudan, Elfasher and Nyala in which the pupils are frequently exposed to the same content

of chemicals and contaminants in the drinking water. High mineral content and bacterial contamination of water may also be goitrogenic. An association has also been found between malnutrition and development of goiter, which may indicate an enhancement of the effect of goitrogens (Gaitan, 1990).

CHAPTER SIX

6.1 CONCLUSIONS

The findings of this study revealed that:

a. Goitre was endemic in the whole studied populations of Sudan
b. Iodine deficiency was noticed throughout the studied areas of the country except in coastal region of Port Sudan city.
c. There was a lack of significant relationship between the urinary iodine excretion and the endemicity of goiter in the population studied suggesting the presence of some goitrogenic factors. Among these was increased consumption of the thiocyanate containing plants, evidenced by the increased secretion of SCN in the urine of subjects from different areas of the country.
d. The presence of high concentrations of some chemical elements in the drinking water specially chloride, calcium and magnesium ions had instrumental role in the goiter etiology.
e. Goiter etiology and thyroid abnormalities are multi-factorial diseases that cannot be easily determined by a single factor since many environmental elements exert different effects at different levels of thyroid hormone production and consequent thyroid anomalies. Accordingly, each city or region should be studied independently as far as goitre is concerned.
f. Determination of serum concentrations of the thyroid related hormones, T4, T3 and TSH is impractical for monitoring iodine nutritional status.
g. Measurements of serum Tg level is more reliable tool for monitoring thyroid iodine status and IDD.

6.2 RECOMMENDATIONS

Monitoring of drinking water for chemical and bacterial contents is recommended. Nutritional studies concerning micronutrients such as iron and

vitamin A as well as protein energy are also recommended in parallel with total goitre rate and iodine nutritional status.

CHAPTER

SEVEN

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