ENDOCRINE AND CLINICAL ASSESSMENT OF THYROIDAL FUNCTION IN PREGNANT MOTHERS AND NEONATES FROM IODINE DEFICIENT REGIONS OF PAKISTAN

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A thesis submitted in partial fulfilment
of the requirements for the degree of
Doctor of Philosophy

Department of Biological Sciences
Quaid-i-Azam University
Islamabad, Pakistan
1997

CERTIFICATE

This thesis by Ms. Riffat Ayesha Anis is accepted in its present form by the Department of Biological Sciences, Quaid-i-Azam University, as satisfying the thesis requirements for the degree of Doctor of Philosophy in Biology (Endocrinology).

Internal Examiner _____

External Examiner _____

External Examiner _____

Chairman _____

Date 30-12-1997

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In the name of Allah the most compassionate and most merciful

ACKNOWLEDGMENTS

I wish to express my deep gratitude to Professor, Dr. M. Arslan for providing me the professional guidance, support, helpful advice and criticism during the course of this study.

I am also obliged to Dr. M. Shahab, Asstt. Professor without whose keen and personal interest, patience and able guidance this work would not have been in its present shape.

My cordial thanks are due to Professor, Dr. M.A. Hafeez, Chairman, Department of Biology, for his kind and helpful attitude throughout my study.

I extend my gratitude to Dr. M. Moti-ur-Rahman Khan, Chief, Nutrition Division, NIH, for his affection, advice and painstaking efforts for helping me out in the clinical studies.

I extend my cordial thanks to Major Gen M.I. Burny (Rtd). Scientist Emeritus, NIH and Col. Farooq A. Khan, Head Dept. of Endocrinology, AFIP, for their valuable suggestions. I am grateful to Dr. A. Ghafoor (late), Dr. Nisar A. Sheikh, Dr. Shahid Abass, NIH, for their support and continuous encouragement. I owe a lot to Mr. Maqsood Ahmed and Ms. Fatima Sughra for their cooperation in carrying out field study and laboratory work successfully.

I feel pleasure to acknowledge the valuable support of Dr. M. Afzal, Director General, PASTIC for his contribution as an expert in the technical matters. Thanks are due to Mr. Zaheer Nasir for the technical support. I also acknowledge the help extended by Mr. Imtiaz, Mr. Asif and Mr. Akram during the typing of this manuscript.

I am grateful to Dr. Talat Rizvi, Dr. Sadaat Aliya, Mr. Waheed-uz-Zaman, Ms. Sarwat Jehan and all other laboratory fellows for their valuable help, support and continuous encouragement.

Thanks are due to all the Director Health Services, District Health Officers, Doctors and the paramedical staff for their cooperation and help in carrying out the field work successfully.

My special thanks are due to patients (women) who showed patience and high-spirit in donating blood, without their cooperation it would not have been possible to undertake this study.

Last but not the least, I am indebted to thank my mother, mother-in-law, sisters, brothers, husband and children for their never ending prayers, love and affection.

Financial support for the first year to conduct this study from World Health Organization, EMRO, Alexandria is highly acknowledged.

ABSTRACT

The present study was undertaken to examine the clinical and endocrine parameters of thyroid in a total of 460 pregnant women belonging to non-goitre areas (group 1; n=156) and endemic areas with no iodine supplementation (group 2; n=154) and iodine supplementation (group 3; n=150), and their respective new-borns. Blood samples were obtained during each trimester and after delivery. Serum triiodothyronine (T_3), thyroxine (T_4) and thyroid stimulating hormone (TSH) levels were measured by specific enzyme immunoassays. Umbilical cord blood samples were obtained at parturition and analyzed for serum T_4 and TSH levels. Women of goitre-endemic areas (group 3) with visible goitre were administered two capsules of iodized oil orally each containing 200 mg of iodine, within 4-6 weeks of pregnancy. The efficacy of iodine supplementation was assessed by observing changes in thyroid hormones, TSH and birth weights of new-borns, and clinically evaluating the regression in the goitre size. There was a significant increase in the T_3, T_4 and TSH levels, throughout pregnancy in all the three groups. However, there was a significant decrease in the hormone levels after the delivery. In group 2 serum T_4 concentrations were low while T_3 and TSH levels were high which showed hypothyroidism in the women of endemic areas. Goitre size decreased in most of the subjects who received a single dose of iodized oil. There was an increase in serum concentrations of thyroid hormones, whereas TSH levels decreased. However, the levels could not become comparable to those observed in women of non-goitre areas (group 1). Iodine-supplementation also resulted in raised T_4 and low TSH levels in the cord-blood of the neonates. The cord-blood serum TSH levels were high when compared with the values of mothers in all the study groups. During the course of study 2 abortions occurred in the women of group 2 and none in the women of group 1 and 3. There was no stillbirth in the women of group 3 while 1 in group 1 and 3 stillbirths in

group 2 women were observed. All the new-borns were normal except one cretin was born in the group 2 women. The present study reveals that the oral administration of a single dose of iodized oil is capable of correcting iodine deficiency both clinically and endocrinologically in mothers and neonates. Iodine supplementation decreases reproductive losses and also has a positive impact on the birth weight of new-borns.

INTRODUCTION

Iodine deficiency is among the major and widespread nutritional deficiencies causing a significant public health problem in the developing world (Anonymous, 1994). It is among the most effectively and cheaply preventable of all nutritional deficiencies. However, despite its widespectrum nature and the fact that simple and easy methods for its eradication have been known for several decades, even today it still continues to affect hundreds of millions of people in Asia, Africa and South America (Hetzel, 1987).

The term iodine deficiency disorders (IDD) was introduced in 1983 and has since become generally accepted. Iodine deficiency disorders refer to the wide spectrum of effects of iodine deficiency on growth and development of individuals. Previously the term goitre had been used as this was the most obvious and familiar feature of the iodine deficiency (Hetzel, 1983). Goitre means a thyroid gland that is bigger than normal while cretinism refers to the very severe consequences of hypothyroidism occurring during fetal or neonatal life. Iodine deficiency disorders are characterized most commonly by mental deficiency (Delong et al., 1985), deaf-mutism (Goslings et al., 1975), spastic diplegia (Hetzel et al., 1988), lesser degrees of neurological defects (Nagataki and Morita,1991), impaired mental function in children and adults (Bleichrodt et al., 1980), increased stillbirths or spontaneous abortions and perinatal and infant mortality (Greenman et al., 1962; Jones and Man, 1969).

The principal aetiological factor in the development of IDD is inadequate intake of iodine. Iodine is an element which is thinly scattered over the surface of the earth and is an essential component of the thyroid hormones produced by the thyroid gland. Human beings as well as animals have the power to extract iodine from the

environment and concentrate it in a defined organ, where it is stored in an organic form. As needed, this stored iodine is secreted into the blood in the form of thyroid hormones.

Normal thyroid function is essential for growth and development. Thyroid hormones include thyroxine (T_4) and triiodothyronine (T_3). Triiodothyronine appears to be more active form of the thyroid hormones (Braverman et al., 1970). Thyroid hormones have many physiological effects by causing alterations in all metabolic pathways and organs. Thyroid hormones regulate oxygen consumption (Haber et al., 1984; Gick et al., 1988), and protein (Burris et al., 1995), carbohydrate (Sestoft, 1980), lipid (Hoppichler et al., 1995) and vitamin (William et al., 1995) metabolism. Thyroid hormones alter the secretion and degradation rates of other hormones and growth factors (Nato-Salonen et al., 1991). In amphibians the process of metamorphosis from tadpoles to frog is strictly dependent upon thyroid hormones (Gudernatsch, 1912; Shellabarger et al., 1959).

The effects of thyroid hormone can broadly be divided into two categories: effects on cellular differentiation and development and, effects on metabolic pathways. These two actions of thyroid hormones are interrelated, in that alterations in growth and development require concomitant shifts in metabolism. Similarly, changes in cellular differentiation can alter the pattern of gene expression by influencing metabolic pathways. Thus, the effects of thyroid hormones represent a complex integration of pathways both at the cellular level and in terms of whole animal physiology.

In humans, thyroid hormones are required for development and their deficiency leads to cretinism (Held et al., 1990). Thyroid hormones continuously play a critical role during growth and development in childhood which is evident in delayed growth curves that occur in hypothyroidism (Mganga, 1990; Sustan-Assin, 1990). In adults,

the primary effects of thyroid hormones are manifested by alterations in metabolism. The clinical feature of hypothyroidism and hyperthyroidism clearly show that thyroid hormones cause pleiotrophic effects that reflect their action on many different pathways and target organs.

One of the hallmarks of thyroid hormone action has been recognised by the alterations in oxygen consumption (Freake and Oppenheimer, 1995). Clinically, this aspect of thyroid hormone action forms the basis for measurements of basal metabolic rate (BMR), which is reduced in hypothyroidism (Duquensnoy et al., 1985), and increased in hyperthyroidism (Torizuka et al., 1995). Oxygen consumption measurements in individual tissues have provided an index of organs that are targets for the thyroid hormone action. The metabolic effects of thyroid hormones are highly variable in different organs. Oxygen consumption is stimulated markedly by thyroid hormones in heart, skeletal muscle, liver, kidney and gastrointestinal organs, whereas brain, spleen and gonadal tissues are less responsive (Baker and Klitgaard, 1952; Baker and Schwartz, 1953). The pituitary gland exhibits a paradoxical response to thyroid hormones with increased metabolic activity in hypothyroidism and decreased activity in hyperthyroidism (Jameson and DeGroot, 1995). The reasons for differential responses of tissues to thyroid hormones are not clear. Variable tissue responses may be contributed by the qualitative nature of the thyroid hormone receptors present in different target tissue.

A number of theories have been proposed to explain the thyroid hormone action. Initially, thyroid hormone was thought to act by uncoupling oxidative phosphorylation. Subsequently, thyroid hormone was proposed to increase energy expenditure by stimulating Na^+, K^+ ATPase activity that causes alterations in gene expression (Brent et al., 1991). Thyroid hormone may also have direct effects on selected transporters and enzymes in the plasma membrane and mitochondria. Most

of the effects of thyroid hormone are now considered to occur through the actions of nuclear receptors that cause alterations in gene expression (Brent et al., 1991).

The control of thyroid hormone synthesis not only involves the thyroid, but the pituitary, the brain and the peripheral tissues also (Dumont and Vassart, 1995). There are two main factors that control the physiology of the thyroid which are the requirement for thyroid hormones and the supply of its main and specialized substrate, iodine. Thyroid hormone plasma levels are monitored by the hypothalamic supraoptic nuclei and the thyrotrops of the anterior lobe of the pituitary. Iodide levels are sensed by the thyroid itself where it inhibit thyroid function and thyrocyte response to thyroid stimulating hormone (TSH). The function and size of the thyroid is controlled by these two major physiological regulators- TSH positively and iodine negatively (Dumont, 1971; Brabant et al., 1992; Dumont et al.,1992; Vassart and Dumont, 1992).

Human chorionic gonadotropin (hCG) at high levels stimulate the thyroid and this effect elevates thyroid activity in pregnancy (Pekonen et al., 1988). Some non specific hormones also influence the thyroid gland e.g. estrogen's affect the thyroid directly or indirectly in the menstrual cycle and pregnancy (Hershman et al., 1988; Glinoer et al., 1990; Hershman, 1992). In vitro and vivo studies also demonstrate the effects of locally secreted neurotransmitters and growth factors on the thyrocytes. However, the set of neurotransmitters acting on the thyrocyte and their effects vary from species to species (Ahren, 1991; Dumont et al., 1991). In human beings well defined direct but short-lived responses of thyroid to norepinephrine, adenosine triphosphate (ATP), adenosine, bradykinin, and thyrotropin-releasing hormone (TRH) have been observed (Van-Sande et al., 1988;. Raspe et al., 1989; Dumont et al., 1991).

Effects of Insulin-like growth factors I (IGF-I), epidermal growth factor (EGF), hepatocyte growth factor (HGF), tumor growth factor (TGF) and fibroblast growth factor (FGF) have also been demonstrated on thyrocytes of human and other species in vitro (Tramontano et al., 1986; William et al., 1989; Dumont, 1991; Dumont et al., 1992). Evidence also suggests that IGF-I may also play a biological role in the growth of the human thyroid gland (Dumont, 1992).

The daily requirement of iodine is estimated to be 100-150 microgram for adults (Hetzel, 1989; Phillips, 1989; Delange, 1994). When the supply of iodine falls below a certain minimum level, iodine deficiency results which constitute a major public health problem (Stanbury, 1987). Toxic action of goitrogenic agents which interfere with metabolism of iodine and formation of thyroid hormones are also important in the causation of IDD. The most important goitrogens are probably thiocyanates, either preformed or synthesized from other compounds, goitrin, flavonoids and very recently selenium deficiency have also been implicated as causes of goitre (Thilly et al., 1993; Konde et al., 1994). Dietary goitrogen are found in a variety of foods such as millet, cassava, soyabean, vegetables of the genus Brassica including cabbage and turnip, and plants of the family Cruciferae (Gaiten, 1980; Woeber, 1991; Delange, 1994) which have toxic action on thyroid development (Ermans et al., 1972). In many areas of endemic iodine deficiency consumption of cassava meals gives rise to thyocinates and aggravates the iodine deficient state by inhibition of thyroid iodide transport (Delange et al., 1982). Contamination of drinking water with Escherichia coli and toxins from other microbes (Gaiten et al., 1971), and with urochrome, one of the products of human waste (Tettche, 1955), has also been implicated as another cause for goitre. In recent years, excess iodine has been shown to be a cause of the enlargement of the thyroid gland (Xian-Yi et al., 1984; Mul et al., 1987). Furthermore, nutritional factors including vitamin A deficiency and protein-energy

malnutrition (PEM) have been reported as important factors in the occurrence of goitre (Koutras et al., 1973; Ingenbleek, 1986).

It has been even demonstrated that in many cases iodine supplementation is sufficient to prevent most manifestations of IDD and, in some instances, even to cause goitre to recede (Eltom et al., 1985; Hetzel et al., 1987; Hintze et al., 1989; Tonglet et al., 1992). Supplementation with iodine to a newborn baby suffering from iodine deficiency, for example, immediately normalizes the hormonal balance and promotes normal and healthy growth (Obregon et al., 1984; Woods et al., 1984).

Iodine deficiency is a major impediment to human development and its social impact is great. The quality of life, productivity and educational intelligence of a community can be improved by preventing of iodine deficiency through iodine supplementation using iodized salt (Tai et al., 1982) or iodized oil (Buttfield and Hetzel, 1967). Salt iodination is a long term measure, but a major method used since 1920s when it was first introduced in Switzerland (Supersaxo et al., 1991). Since then, successful programs have been carried out in Central and South America, Europe and Asia (Stanbury and Hetzel, 1980).

Iodized oil has been used as a short term measure in many parts of the world to combat iodine deficiency. Iodized oil injections were first used in New Guinea to prevent endemic goitre and endemic cretinism (McCullagh, 1963). Further studies were done in South America (Hetzel et al., 1980), Indonesia (Djokomoeljanto et al., 1983) and China (Tai et al., 1982). The iodized oil is given by intramuscular injection in a dose of 1 ml which contains 480 mg of iodine. Absorption of iodine from the preparation into the blood occurs slowly as it remains at the site for weeks or months. Once in the blood stream the iodine can be taken up by the thyroid, excreted in the urine, and stored in the fatty tissues of the body. Treatment can

correct severe iodine deficiency for a period of four to five years (Dunn, 1986; Woeber, 1991). The disadvantages of the injections are the immediate discomfort produced and infrequent development of abscesses at the site of injection.

Oral iodized oil is deionized in the liver and other tissues and the iodine is taken up by the thyroid or excreted by the kidney, the rest is incorporated into fatty tissues. The iodine stored in the fat is then slowly released. The oral administration is effective for one to two years. (Wantanabe et al ., 1974; Eltom et al., 1985; Tonglet et al., 1992). The main hazard of iodination is transient thyrotoxicosis, observed mainly in women over the age of 45 (Watanabe et al., 1974; Maberly et al., 1982; Hetzel, 1983; Woeber, 1991).

Goitre and cretinism have been reported in northern areas of Pakistan as early as beginning of the twentieth century (McCarrison, 1908). In Pakistan several surveys about goitre, reporting the incidence of cretinism as well, have been conducted during the last two decades (Anonymous, 1970; Ali and Khan, 1976; Mahmud et al., 1978). They indicate high and sometimes, alarming prevalence of endemic goitre and cretinism in certain regions. The northern most areas of Pakistan are among the most severely endemic areas in the world, with goitre prevalence there varying between 70 and 80 percent. The severity of iodine deficiency decreases to mild or moderate endemicity towards the south with 15-40 percent prevalence in the hilly areas that border the plains. The North-West frontier province of Pakistan has areas with high to moderate endemicity (Bagchi and Rejeb, 1987). The iodine deficiency disorder is also endemic in other areas and pockets have been identified in Punjab and Sind provinces (Rajput et al., 1994).

Although, incidence of goitre in the Northern areas of Pakistan and other parts of the country is well studied (McCarrision, 1908; Chapman et al., 1972; Zafar et al., 1985;

Khan et al., 1987; Lakhani et al ., 1989; Khan et al., 1990; Stewart, 1990; Zafar et al., 1991; Ali et al., 1992), very little is known about endocrine attributes of hypothyroidism in these areas. Relatively few data are available on secretion of thyroid hormones but that too in normal subjects (Akhter et al., 1986) or under pathological conditions (Tayyab et al., 1993). In one study, however, endocrine assessment of thyroid function has been reported for individuals resident in a relatively iodine deficient area (Ali et al., 1992). In view of lack of basal endocrine data on thyroid function in known goitre endemic areas of Pakistan, the present study was initiated to characterize dynamics of thyroid hormones in such areas. Since, iodine deficiency has detrimental effects on fetal development (Vermiglio et al,. 1995), in the current study thyroid hormone and TSH secretion alongwith clinical assessment of the thyroid were systematically investigated longitudinally during the pregnancy and following delivery in endemic areas with and without iodine supplementation. Endocrine assessment of thyroid hormones were also carried out in the new born. For comparative purposes assessment of thyroid hormones during pregnancy and after birth, and neonatal growth and the hormone concentrations were also monitored in non-goitre areas.

MATERIALS AND METHODS

A total of 490 pregnant women of lower socio-economic status belonging to goitre-endemic and non-goitre areas was studied. Informed consent was obtained from each subject. (Annexure 1). Name, age and place of residence of each subject was recorded. Thirty women left the study for various reasons leaving 460 women who were followed until after delivery. Anthropometric measurements i.e. weight and height of subjects were recorded using bathroom scale and fibre glass measuring tape (Table 1). Hemoglobin levels of the subjects were determined in the field using HemoCue B-Hemoglobin Fotometer (HemoCue AB, Angelholm, Sweden; Table 2). Particular emphasis was placed on clinical evidence of hypo- or hyperthyroidism. The presence or absence of goitre was determined by palpation of the thyroid gland with the finger tips of both hands. The size of the thyroid gland increases with the formation of a goitre. Enlargement is regarded significant in the human when the size of the lateral lobe of thyroid gland is greater than the terminal phalanx of the thumb of the person examined. The thyroid gland was examined, using methods described by Perez et al. (1960) and was classified as follows: Stage 0 absence of goitre; stage 1 goitre palpable, but visible only when the neck is fully extended; stage 2 goitre easily visible when the neck is in normal position; and stage 3 very large goitre (Annexure 2). The subjects were divided into three groups:

1. Group 1 consisted of 156 subjects (mean ± SEM age: 25.30±1.50 years) of non-goitre areas of Rawalpindi. All women were clinically in euthyroid status and had no previous known thyroid disorders. Women of this group had history of a total number of 8 spontaneous abortions, 3 stillbirths and 3 abnormal children with squint eyes/stunted growth in the family. This group served as a control for the whole study.

2. Group 2 consisted of 154 subjects (mean ± SEM age: 25.7±1.51 years) of the goitre-endemic areas of Swat, Muzaffarabad and Skardu with and without thyroid enlargement. Among these women 25 were in stage 0, 72 in stage 1 and 57 in stage 2 of the goitre size. The women in stage 3 were not included in the study. Women of this group had history of a total number of 19 spontaneous abortions, 13 stillbirths and 9 abnormal children in the family. This group was not given any treatment and served as a non-supplemented control group for endemic areas.

3. Group 3 consisted of 150 subjects (mean ± SEM age: 26.8±1.54 years) of endemic areas of Swat, Muzaffarabad and Skardu with thyroid enlargement upto stage 2. Sixty-eight women were in stage 1 and 82 were in stage 2. These women were given a single dose of two capsules of iodized oil (iodized ethyl esters of poppy seed oil; Lipoidal Ultrafluid, 400 mg of iodine, Guerbet Laboratories, Paris, France) orally in the first trimester within 4-6 weeks of pregnancy. Iodine supplementation caused thyrotoxicosis in 4 women, the clinical signs observed were tachycardia, a well known condition of iodine induced thyrotoxicosis called Jod-basedow phenomenon. These women were excluded from the study. Few women reported submandibular swelling and pain after the administration of iodized oil which subsided within 3-4 days. The submandibular glands other than thyroid concentrate iodine at a level above the serum concentration and are prone to adenitis (Follis, 1963).The women of this group had history of a total number of 21 spontaneous abortions and 11 stillbirths. The families had 6 abnormal children and 2 neurological cretins. This group of subjects constituted the treatment study group.

Pregnant women were identified with the help of birth attendants or lady health visitors of the particular areas. In some cases pregnancy tests were also conducted on the spot by measuring gonadotropin concentrations in urine by direct agglutination method (Betatex Direct; Omega Diagnostics Limited, Alloa, United Kingdom).

Blood samples were obtained in disposable syringes during each trimester for quantification of thyroid hormones and TSH. The sampling times for each trimester were: first trimester: 10 to 13 weeks, median 12 week; second trimester: 15-18 weeks, median 16 week; third trimester: 26-38 weeks, median 32 week. Post-partum blood samples were obtained between 6-8 weeks after the delivery. Blood samples were centrifuged at 4000 g for 10 minutes within 2 hours of blood collection. Serum was separated, frozen immediately and stored at -70°C until analysed.

Obstetric histories of the subjects were taken (Table 4). Information about normal or abnormal children in the family were noted. Outcome of the present pregnancies were observed. Neonates were examined for clinical signs of cretinism and their birth-weights were recorded using baby-weighing scales (Annexure 2). Umbilical cord blood samples were taken immediately at parturition, sera separated and stored at -70°C. Serum was analyzed for T_4 and TSH concentrations by specific EIA.

Serum concentrations of T_3, T_4 and TSH were measured by using specific enzyme immunoassays (EIA). The hormone concentrations were measured using the EIA kits manufactured by Serono Diagnostic SA, Coinsins, Switzerland. (Annexure 3,4, 5). All hormone determinations were made in duplicate. The sensitivity for the T_3, T_4 and TSH assay was 0.15 ng/ml, <5 ng/ml and 0.03 μIU/ml, respectively. Intra- and inter-assay coefficients of variation were for T_3 3.1% and 4.3%, for T_4 2.9% and 4.1% and for TSH 2.1% and 4.7 %, respectively.

The results were analyzed using standard statistical procedures. Mean concentrations of thyroid hormones and TSH as well as birth weights were analyzed through ANOVA using MSTAT-C program and were further separated employing Duncan's multiple range test. Reduction in goitre size in iodine supplemented subjects was analyzed by Chi-square test. All statistical inferences were drawn at $p<0.05$.

Table 1. Anthropometric data of the women studied.

PARAMETER	GROUP 1	GROUP 2	GROUP 3
Height (cm)	156.5±5.9[a]	155.2±4.4	154.8±4.3
	(148-176)[b]	(146-171)	(144-170)
Weight (kg)	49.2±4.2	48.6±3.6	48.1±3.0
	(40-61)	(39-58)	(38-59)

[a] Each value represents mean ± SEM

[b] Values in parenthesis represent the range.

Table 2. Hemoglobin status of the women studied.

INDICATOR	GROUP 1	GROUP 2	GROUP 3
Hemoglobin (g/dl)	9.86±1.31[a] (7-12.5)[b]	10.00±1.39 (8-13.4)	10.18±1.27 (8-13.0)

[a] Each value represents mean ± SEM.

[b] Values in parenthesis represent the range.

RESULTS

Changes in mean serum concentrations of thyroid hormones (T_3, T_4) and TSH during the pregnancy and post-partum period in normal, iodine deficient and iodine supplemented women are illustrated in Figure 1-3.

In group 1 (control group) serum levels of T_3 changed markedly ($P< 0.001$) during pregnancy and post-partum period (Fig. 1). Concentrations of T_3 averaged 1.27±0.05 ng/ml during the first trimester which increased significantly ($P<0.05$) to 1.48±0.06 ng/ml in the second trimester. Maximum T_3 (1.56±0.06 ng/ml; $P<0.05$) concentrations were attained during the third trimester. Concentrations of T_3 decreased dramatically ($P<0.05$) after the birth and averaged 0.98±0.04 ng/ml which were significantly ($P<0.05$) lower than the values observed during the pregnancy.

In group 1 serum T_4 levels also changed markedly ($P<0.001$) during pregnancy and post-partum period (Fig. 2). T_4 concentrations averaged 99.24±2.99 ng/ml during the first trimester which increased significantly ($P<0.05$) to 107.82±2.98 ng/ml in the second trimester. In the third trimester there was no further ($P>0.05$) change in concentrations of T_4. Concentrations of T_4 decreased dramatically ($P<0.05$) after the birth and averaged 85.88±2.9 ng/ml which were significantly ($P<0.05$) lower than the values observed during the pregnancy.

Serum levels of TSH changed markedly ($P<0.001$) during pregnancy and post-partum period in group 1 women (Fig. 3). Concentrations of TSH averaged 2.36±0.27 μIU/ml during the first trimester which increased significantly ($P<0.05$) to 2.54±0.25 μIU/ml in the second trimester. Maximum TSH (3.42±0.34 μIU/ml; $P<0.05$) concentrations were attained during the third trimester. Concentrations of

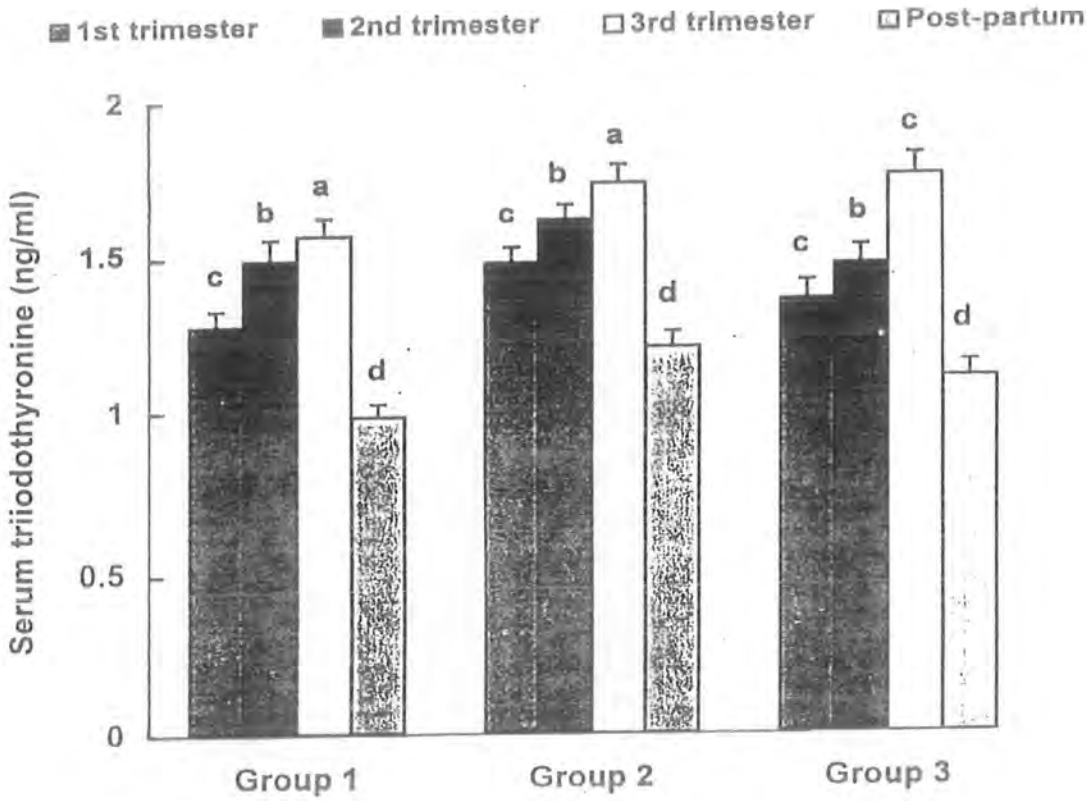

Fig. 1. Serum concentrations (Mean±SEM) of T_3 in women of non-endemic (Group 1; n=156) and endemic-areas with (Group 3; n=150) and without (Group 2; n=154) iodine supplementation, during pregnancy and after delivery. Concentrations of T_3 varied significantly at various trimesters and post-partum periods in the study groups ($P<0.001$). Significant ($P<0.05$) intra-group comparisons for mean T_3 concentrations at various trimesters and post-partum period are indicated by different letters above the bars.

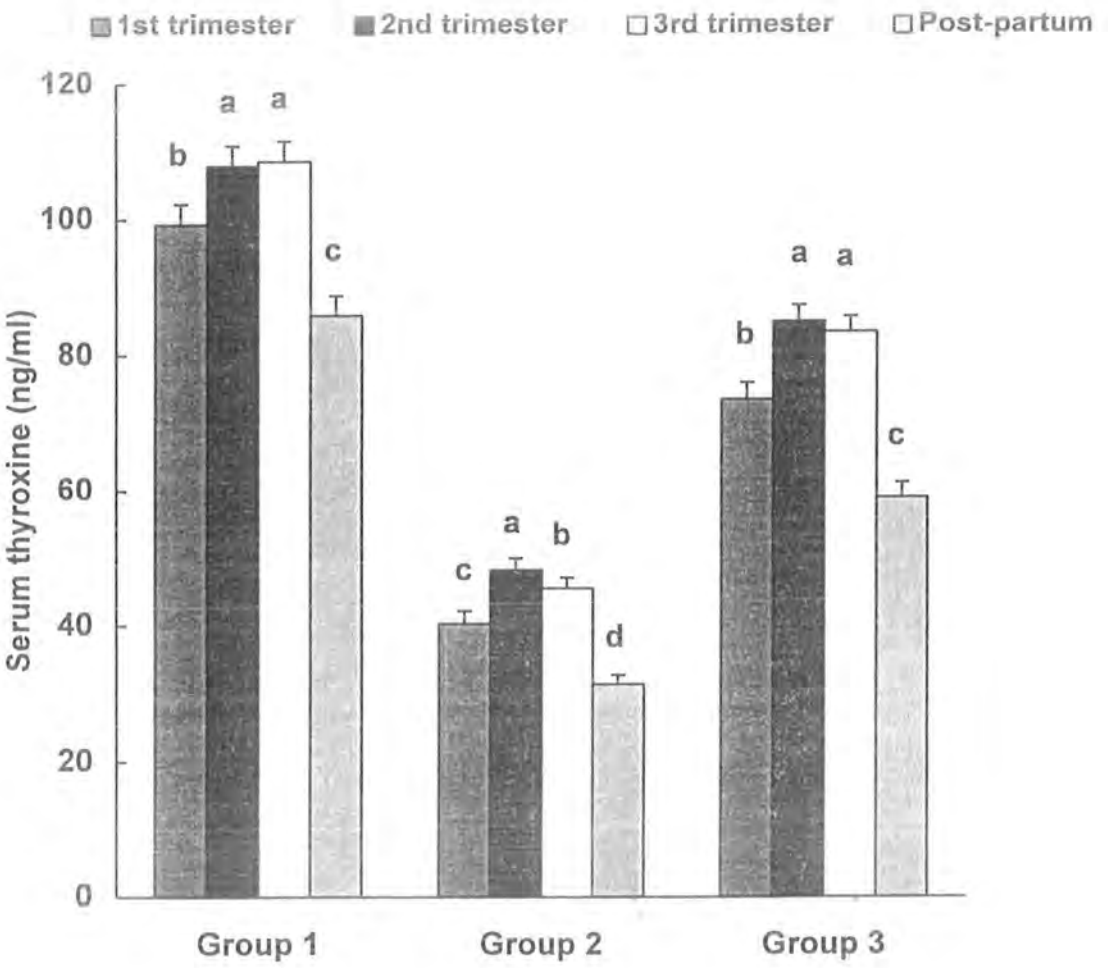

Fig. 2. Serum concentrations (Mean±SEM) of T_4 in women of non-endemic (Group 1; n=156) and endemic-areas with (Group 3; n=150) and without (Group 2; n=154) iodine supplementation, during pregnancy and after delivery. Concentrations of T_4 varied significantly at various trimesters and post-partum periods in the study groups ($P<0.05$-0.001). Significant ($P<0.05$) intra-group comparisons for mean T_4 concentrations at various trimesters and post-partum period are indicated by different letters above the bars.

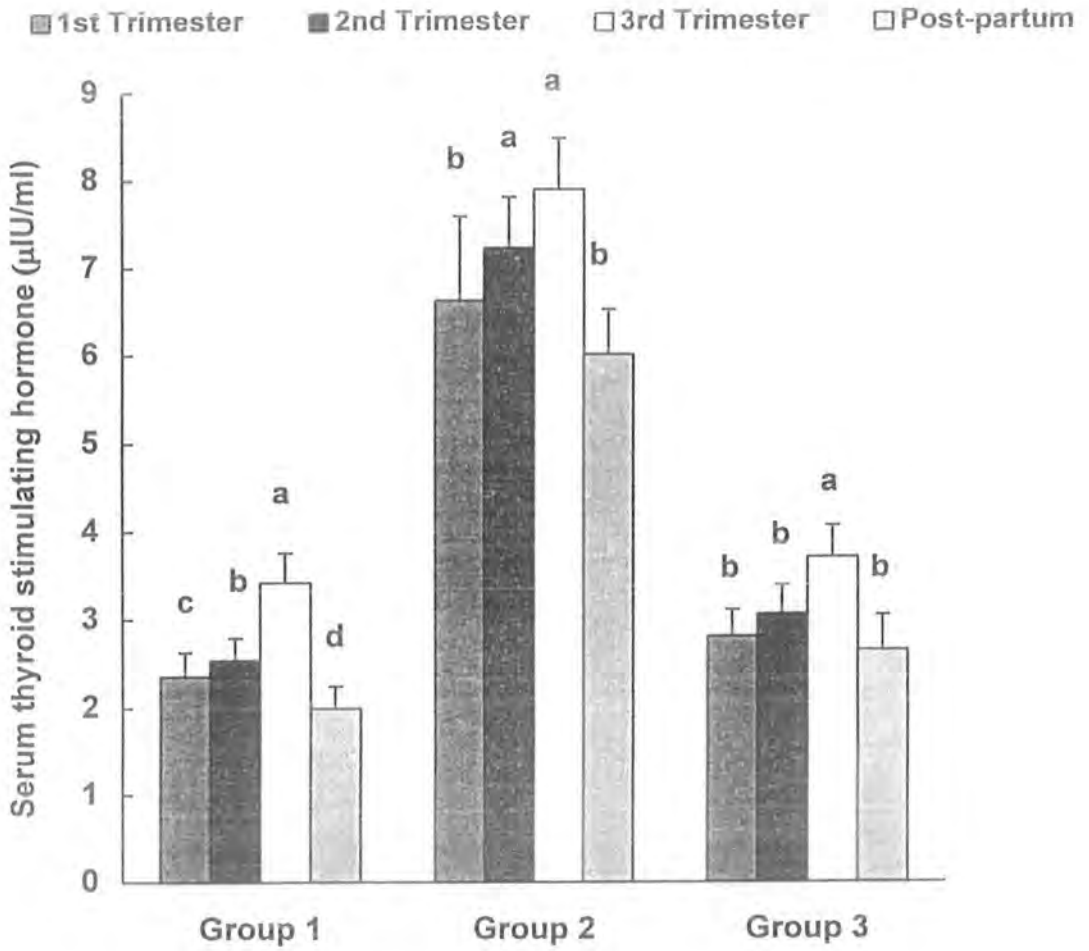

Fig. 3. Serum concentrations (Mean±SEM) of TSH in women of non-endemic (Group 1; n=156) and endemic-areas with (Group 3; n=150) and without (Group 2; n=154) iodine supplementation, during pregnancy and after delivery. Concentrations of TSH varied significantly at various trimesters and post-partum periods in the study groups ($P<0.001$). Significant ($P<0.05$) intra-group comparisons for mean TSH concentrations at various trimesters and post-partum period are indicated by different letters above the bars.

TSH decreased abruptly ($P<0.05$) after the birth and averaged 2.00±0.25 μIU/ml which were significantly ($P<0.05$) lower than the values observed during pregnancy.

In group 2 (iodine-deficient areas), serum levels of T_3 changed markedly ($P<0.001$) during pregnancy and post-partum period (Fig. 1). Concentration of T_3 averaged 1.47±0.05 ng/ml during the first trimester which increased significantly ($P<0.05$) to 1.61±0.05 ng/ml in the second trimester. Maximum ($P<0.05$) T_3 concentrations (73±0.06 ng/ml) were attained during the third trimester. Concentrations of T_3 decreased to minimal values after the birth and averaged 1.20±0.06 ng/ml which were significantly ($P<0.05$) lower than the values observed during the pregnancy.

Serum concentrations of T_4 in group 2 also changed markedly ($P< 0.005$) during pregnancy and post-partum period (Fig. 2). Thyroxine concentrations averaged 40.39±1.83 ng/ml during the first trimester which increased significantly ($P<0.05$) to 48.32±1.67 ng/ml in the second trimester. In the third trimester T_4 concentrations decreased ($P<0.05$) to 45.58±1.60 ng/ml. Concentrations of T_4 decreased significantly after the birth and averaged 31.44+1.38 ng/ml which were significantly ($P<0.005$) lower than the values observed during the pregnancy.

Serum levels of TSH in group 2 differed markedly ($P<0.001$) during pregnancy and post-partum period (Fig. 3). Thyroid stimulating hormone concentrations averaged 6.63±0.96 μIU/ml during the first trimester which increased significantly ($P<0.05$) to 7.23±0.58 μIU/ml in the second trimester. There was no further increase in TSH levels during the third trimester. Concentrations of TSH decreased ($P<0.05$) after the birth and averaged 6.01±0.52 μIU/ml which were comparable to the values observed during the first trimester.

In group 3 (iodine-supplemented) subjects of endemic areas, serum levels of T_3 changed markedly ($P<0.001$) during pregnancy and post-partum period (Fig. 1). Concentration of T_3 averaged 1.35±0.06 ng/ml during the first trimester which increased significantly ($P<0.05$) to 1.46±0.06 ng/ml in the second trimester. Maximum T_3 1.75 ng/ml ($P<0.05$) concentrations were attained during the third trimester. Concentrations of T_3 dropped dramatically ($P<0.05$) after the birth and averaged 1.10±0.05 ng/ml which were significantly ($P<0.05$) lower than the values observed during the pregnancy.

In group 3, serum T_4 levels varied significantly ($P<0.001$) during pregnancy and post-partum period (Fig. 2). Thyroxine concentrations averaged 73.61±2.43 ng/ml during the first trimester which increased significantly ($P<0.05$) to 85.08±2.35 ng/ml in the second trimester. Thyroxine levels observed during the third trimester (83.51±2.25 ng/ml) were comparable to those of the second trimester. However, there was a dramatic decrease in T_4 concentrations ($P<0.05$) after the birth and the values averaged 59.08±2.21 ng/ml which were significantly ($P<0.05$) lower than the values observed during the pregnancy.

Serum levels of TSH in group 3 also increased markedly ($P<0.001$) during pregnancy (Fig 3). Thyroid stimulating hormone concentrations averaged 2.81±0.30 μIU/ml during the first trimester which increased insignificantly ($P>0.05$) to 3.06±0.33 μIU/ml in the second trimester. Maximum TSH levels (3.71±0.36 μIU/ml) were attained in the third trimester which were greater ($P<0.05$) than the values observed in the second and first trimester. Concentrations of TSH decreased significantly ($P<0.05$) after the birth and averaged 2.65±0.40 μIU/ml.

Comparison of mean serum concentrations of T_3, T_4 and TSH, during various trimesters and after delivery in the women of different study groups, is shown in Figure 4-6.

Serum T_3 levels in the first, third trimester and post partum varied significantly ($P<0.05$) between different groups, while in the second trimester T_3 levels were not different in the three groups (Fig. 4).

Mean T_3 serum concentrations during first trimester observed in group 1 averaged 1.27±0.05 ng/ml which were less ($P<0.05$) than those observed in the group 2 (1.47±0.05 ng/ml). Mean T_3 concentrations in the group 3 (1.35±0.06 ng/ml) were comparable to the levels in group 1 and group 3.

Mean T_3 serum concentrations during second trimester observed in group 2 averaged 1.61±0.05 ng/ml which were slightly higher ($P>0.05$) than those in the other groups (1.48±0.07 ng/ml and 1.46±0.06 ng/ml in group 1 and 3, respectively).

Mean T_3 serum concentrations during third trimester observed in group 1 averaged 1.56±0.06 ng/ml which were less ($P<0.05$) than those observed in group 3 (1.75±0.07 ng/ml) but comparable to the levels in group 2 (1.73±0.06 ng/ml). Mean T_3 serum concentrations in group 2 and group 3 were also comparable.

Post-partum concentrations of T_3 averaged 1.20±0.05 ng/ml in group 2 which were higher ($P<0.05$) as compared to the concentrations in the group 1 (0.98±0.04 ng/ml) but similar to the concentrations in group 3 (1.10±0.05 ng/ml). Mean T_3 concentrations in group 3 and group 1 were not different.

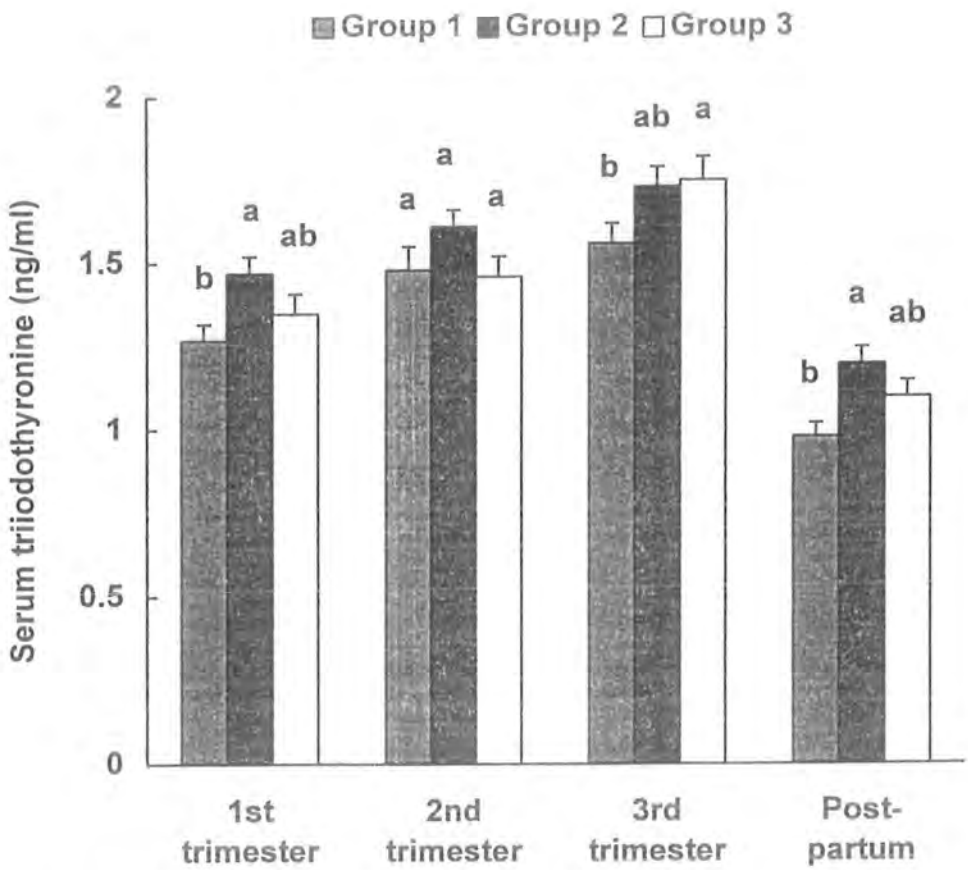

Fig. 4. Serum concentrations (Mean±SEM) of T_3 in women of non-endemic (Group 1; n=156) and endemic-areas with (Group 3; n=150) and without (Group 2; n=154) iodine supplementation, during pregnancy and after delivery. Concentrations of T_3 varied significantly between groups at various trimesters and post-partum periods except that at 2nd trimester ($P<0.05$). Significant ($P<0.05$) inter-group comparisons for mean T_3 concentrations at a given period are indicated by different letters above the bars.

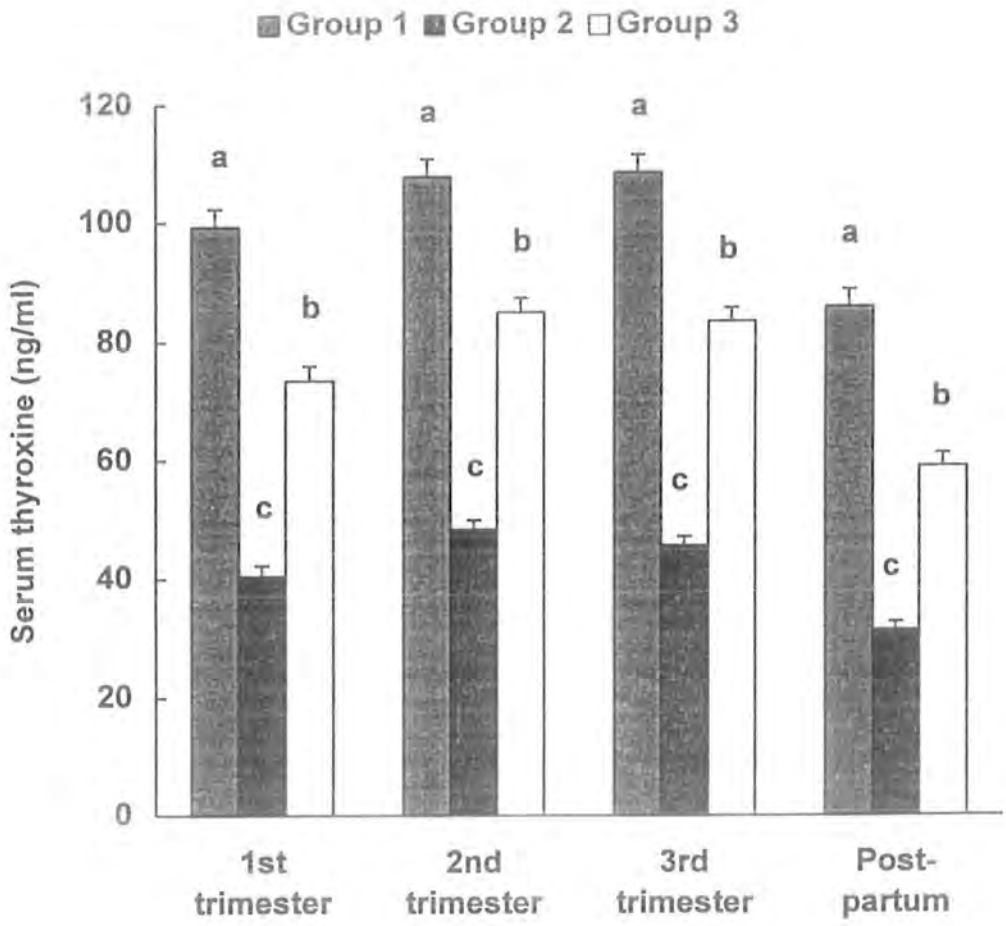

Fig. 5. Serum concentrations (Mean±SEM) of T_4 in women of non-endemic (Group 1; n=156) and endemic-areas with (Group 3; n=150) and without (Group 2; n=154) iodine supplementation, during pregnancy and after delivery. Concentrations of T_4 varied significantly between groups at various trimesters and post-partum periods in the study groups ($P<0.001$). Significant ($P<0.05$) inter-group comparisons for mean T_4 concentrations at a given period are indicated by different letters above the bars.

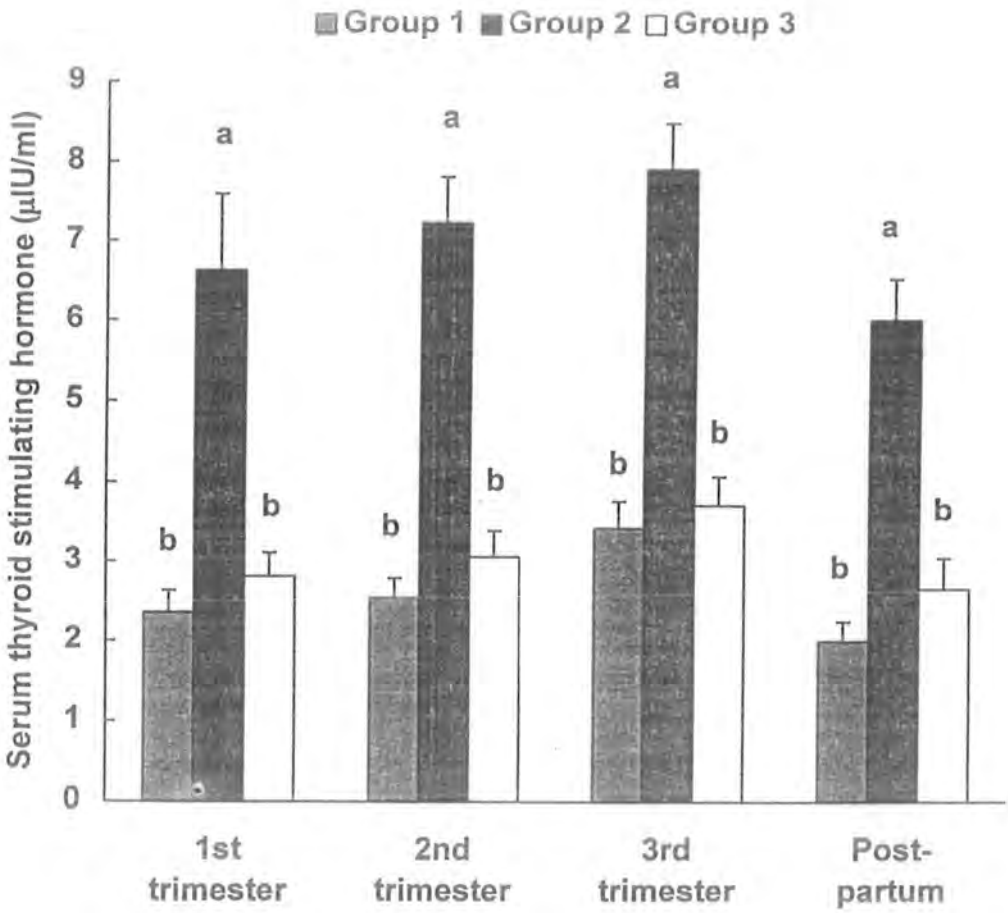

Fig. 6. Serum concentrations (Mean±SEM) of TSH in women of non-endemic (Group 1; n=156) and endemic-areas with (Group 3; n=150) and without (Group 2; n=154) iodine supplementation, during pregnancy and after delivery. Concentrations of TSH varied significantly between groups at various trimesters and post-partum periods in the study groups ($P<0.001$). Significant ($P<0.05$) inter-group comparisons for mean TSH concentrations at a given period are indicated by different letters above the bars.

Serum T_4 concentrations in the first, second, third trimester and post-partum period varied significantly ($P<0.01$ - <0.05) between the three groups (Fig. 5).

The maximum T_4 concentrations (99.25±2.99 ng/ml) during first trimester were observed in group 1 which were significantly ($P<0.05$) higher than the values in group 3 (73.62±2.43 ng/ml) and group 2 (40.40±1.83 ng/ml). Mean serum T_4 concentrations in the group 2 were significantly less ($P<0.05$) than those in group 3.

Concentrations of T_4 were maximum (107.82±2.35 ng/ml) during second trimester in the group 1 which were significantly ($P<0.05$) higher than the values in group 3 (85.08±2.35 ng/ml) and group 2 (48.32±1.67 ng/ml). Mean T_4 concentrations in group 2 were less ($P<0.05$) than those in group 3.

Maximum T_4 concentrations (108.54±3.0 ng/ml) during third trimester were observed in group 1 which were significantly ($P<0.01$) higher than the values in group 3 (83.51±2.25 ng/ml) and group 2 (45.58±1.6 ng/ml). Mean serum T_4 concentrations in the group 1 were significantly less than those observed in group 3.

Highest post-partum T_4 concentrations were observed in group 1 (85.88±2.90 ng/ml) which were significantly greater than the values in group 3 (59.08±2.21 ng/ml) and group 2 (31.45±1.38 ng/ml). Mean T_4 levels were lowest in group 2 women which were also significantly less ($P<0.05$) than those in group 3.

Serum concentrations of TSH observed during various trimesters varied significantly ($P<0.001$) between the groups, while in post-partum period the difference was non-significant between group 3 and 1 (Fig. 6).

Concentrations of TSH in group 2 (6.63±0.96 μIU/ml) during first trimester were significantly ($P<0.05$) higher than those in group 1 (2.36±0.27 μIU/ml) and group 3 (2.81±0.30 μIU/ml). Mean TSH levels in group 1 and group 3 were comparable.

Serum TSH concentrations in group 2 (7.23±0.58 μIU/ml) during second trimester were significantly ($P<0.05$) higher than those in group 1 (2.54±0.25 μIU/ml). Mean TSH levels in group 1 and 3, however, were comparable.

Concentrations of TSH in group 2 (7.90±0.58 μIU/ml) during third trimester were significantly ($P<0.05$) higher than those in group 1 (3.42±0.34 μIU/ml) and group 3 (3.71±0.36 μIU/ml). Mean TSH levels in group 1 and group 3 were comparable.

Post-partum TSH concentrations were higher in group 2 (6.01±0.52 μIU/ml) women which were greater ($P<0.05$) than the values observed in groups 3 (2.65±0.04 μIU/ml) and group 1 women (2.0±0.25 μIU/ml). Mean post-partum concentrations in the group 3 and group 1 women were comparable.

In the cord-blood of neonates mean T_4 levels varied significantly ($P<0.001$) between different groups (Fig. 7).

In the iodine-deficient regions (group 2) mean T_4 levels in the neonates were significantly ($P<0.05$) lower than those observed in the group 1 and group 3. In the endemic areas, neonates whose mother had received oral iodized-oil (group 3) had higher T_4 levels than those whose mothers were not treated with iodized-oil.

Cord-blood levels of TSH in neonates also differed ($P<0.001$) between the study groups. The mean cord-blood TSH levels were the highest in the group 2

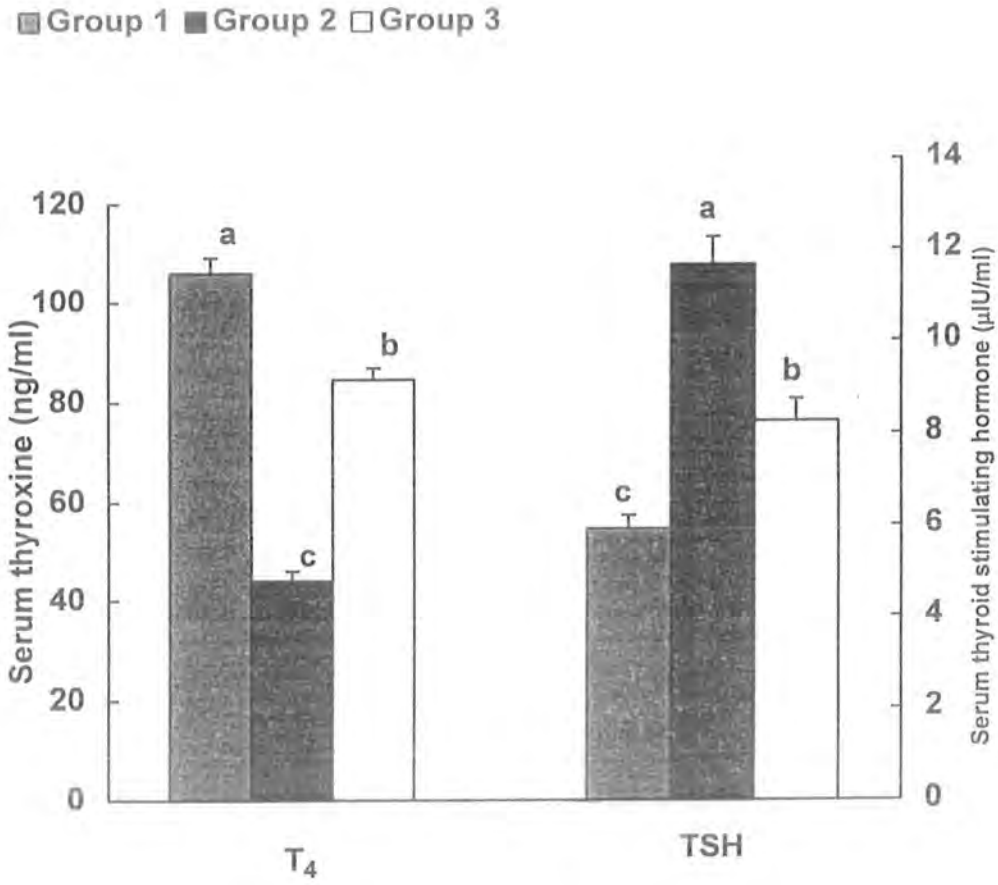

Fig. 7. Mean±SEM serum concentrations of T_4 and TSH in the cord-blood of neonates from women of 3 study groups (n=149, 148 and 146 for group 1, 2 and 3, respectively). Concentrations of T_4 and TSH in neonates varied significantly ($P<0.001$) between groups. Different letters above the bars indicate significant difference ($P<0.05$) between the respective groups for T_4 and TSH.

(11.66±0.61 μIU/ml). Iodine administration to the mothers of endemic areas (group 3) resulted in a significant ($P<0.05$) decline in the mean TSH cord-blood levels (8.26±0.48). The lowest ($P<0.05$) mean cord-blood TSH levels were found in the neonates of group 1 (5.92±0.29 μIU/ml).

Maternal and neonatal concentrations of T_4 were comparable ($P>0.05$) in each group. However, cord-blood TSH concentrations were significantly ($P<0.05$) greater than the maternal levels during the third trimester, in all groups (5.92±0.29 Vs 3.42±0.34, 11.66±0.61 Vs 7.90±0.58 and 8.26±0.48 Vs 3.71±0.36 μIU/ml for group 1, 2 and 3 respectively).

Birth weights of neonates from the different groups are shown in Figure 8. The mean birth weight varied significantly ($P< 0.001$) between the three groups. As compared to the group 1 and group 3 mean neonatal body weights were significantly ($P< 0.05$) low in the group 2 (Fig. 8). However, there was no significant difference between the birth weights of group 1 and group 3 neonates.

During the course of study a total number of 1 neurological cretin was born in the women from goitre-endemic areas (group 2). No cretins were observed in group 1 and group 3.

Regression observed in the size of goitre during the period of study in the women of group 3 is shown in Table 3.

Obstetric problems encountered during the course of study in the women of all the three groups are given in Table 4.

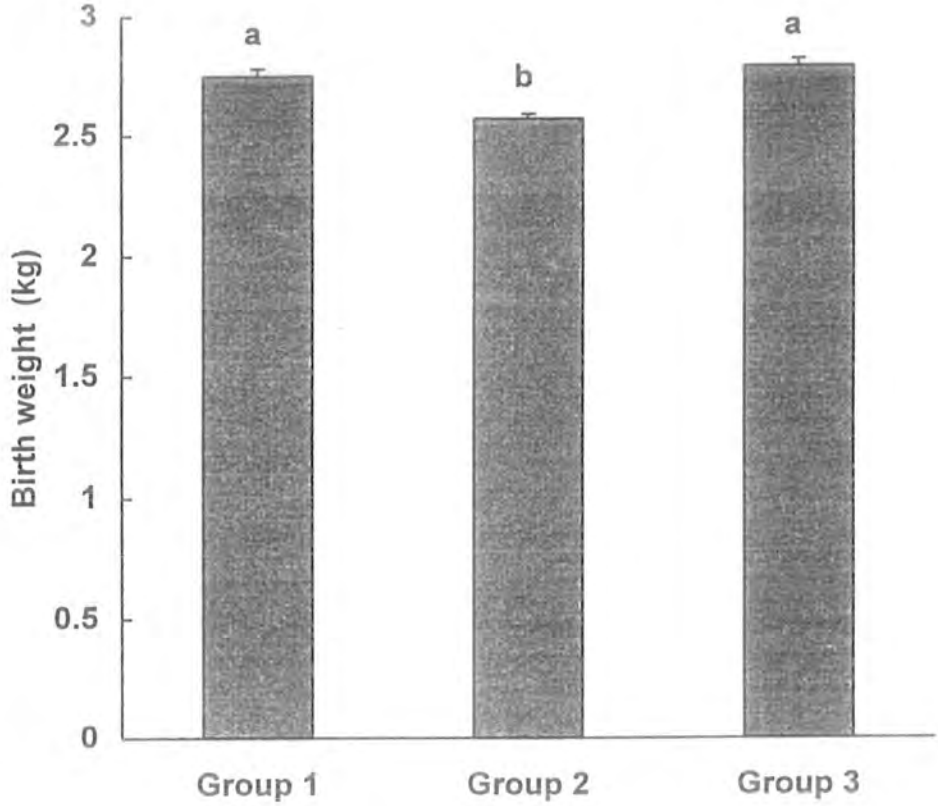

Fig. 8. Mean±SEM birth weights of neonates from women of the 3 study groups (n=140, 140 and 139 for group 1, 2 and 3, respectively). Different letters above the bars show significant difference ($P<0.05$) in birth weights of the respective groups.

Table 3. Effects of iodized oil administration on regression of goitre size in group 3 (n=150)

GOITRE STAGE	BEFORE TREATMENT	AFTER TREATMENT	PERCENT IMPROVEMENT
1	68	47	31 [a] *
2	82	56	32 [b] *

[a] Unquantified improvement.

[b] Conversion of stage 2 to stage 1.

* P<0.001, Chi-square test.

Table 4 . Obstetric history of the women of different study groups.

	NON-ENDEMIC AREAS	ENDEMIC AREAS	
Parameter	**Group 1**	**Group 2**	**Group 3**
No of women	156	154	150
No of spontaneous abortions during the study	0	2	0
No of stillbirths during the study	1	3	0
Sex of the child born during the study			
Males	79	70	78
Females	76	81	72

DISCUSSION

The present study describes systematically the profiles of thyroid hormones and TSH during pregnancy and post-partum period in women from the areas of Pakistan with and without iodine deficiency. In addition, the present study also demonstrates the effect of exogenous iodine administration on the plasma concentrations of thyroid hormones and TSH in pregnant mothers and their newborns in goitre-endemic areas.

In the present study, similar secretory patterns of T_3, T_4 and TSH were evident during pregnancy in women of the 3 study groups. Concentrations of serum T_3 and T_4 were observed to increase during pregnancy. The levels of these hormones increased during first trimester and remained high during the second and third trimester and returned to normal within 6-8 weeks post-partum. Previous studies have indicated that the concentration of thyroid hormones and thyroxine binding proteins (TBG) in serum and the metabolism of iodine change markedly during pregnancy but returned to normal non-pregnant concentrations within 2-3 months after delivery (Robbins and Nelson, 1958; Rebound et al., 1963; Aboul-Khair et al., 1964; Malkasian and Mayberry, 1970; Rastogi et al., 1974; Avruskin et al., 1976; Feely, 1979; Bachrach and Burrow, 1985; Guillaume et al., 1985; Iwatani et al., 1987; Drews et al., 1994). The increased thyroid activity during pregnancy is also indicated by the histological evidence of the large follicles of the thyroid gland filled with abundant colloid, which help in the active formation and secretion of the thyroid hormones (Burrow, 1975).

Several possibilities exist for causation of pregnancy-related increases in thyroid hormone secretion. According to one notion elevation of serum levels of thyroid hormones during pregnancy has been suggested to be due to estrogen induced

increases in TBG (Dowling et al., 1956; Robbins and Nelson, 1958; Feely, 1979; Weeke et al., 1982; Lazarus, 1994). The increased concentration of TBG during pregnancy may contribute to the increased thyroid hormone levels in the pregnant women (Mestman et al., 1969; Ekins, 1978; Rudorff et al., 1978; Boss and Kingstone, 1981).

A number of thyroid stimulators are secreted by placenta including hCG although the exact nature of the placental thyroid stimulators is not known (Feely, 1979; Amir et al., 1980; Price et al., 1989; Lazarus, 1994). Increased thyroid hormone levels during early pregnancy could also be due to thyroid stimulation by hCG, the concentration of which attains its maximum during 10-13 weeks of gestation (Delange et al., 1972; Price et al., 1989; Becks and Burrow, 1991).

In this study, serum levels of TSH in all the groups increased gradually towards the end of the pregnancy also showing its maximum during the later stages. This finding is similar to those of others (Burrow, 1978; Rudorff et al., 1978; Harada et al., 1979; Skjoldebrand et al., 1982; Weeke et al., 1982; Price et al., 1989; Lazarus, 1994).

During pregnancy the increase in serum TSH concentrations reflects the state of increased demand for thyroid hormones (Price et al., 1989; Mandel et al., 1990). Another possibility is that hormonal and metabolic alterations which occur during normal pregnancy, one way or other dampen the normal feed back regulation of the thyroid function either at thyroidal or at the hypothalamic/pituitary level resulting into elevated TSH concentrations (Weeke et al., 1982).

An obvious finding of the current study was that in the endemic-areas plasma concentrations of T_4 were suppressed and that of T_3 and TSH elevated as compared to those in non-endemic-areas. These findings illustrate hypothyroidism in women of

the endemic-areas. The findings of the several previous reports also indicated that subjects living in an area of chronic iodine deficiency had higher or unaffected serum T_3 concentrations as compared to the population residing in non-goitre endemic areas (Dlange et al., 1972; Kochupallai et al., 1973; Patel, 1973; Goslings et al., 1977; Stevenson et al., 1974; Chopra et al., 1975; Medeiros-Neto et al., 1975; Silva and Silva, 1981; Ali et al., 1992). The unchanged T_3 secretion in endemic areas may be ascribed to enhanced synthesis of T_3 in the thyroid gland which occurs in response to decreased peripheral T_3 production due to the less availability of T_4 in iodine-deficient conditions (Silva, 1972; Silva et al., 1974). In this regard it is interesting to mention that T_3 concentrations are higher in subjects with larger goiters (Roti et al., 1986).

The high T_3 concentrations in the pregnant women with goitre is associated with elevated TSH levels (Mediros-Neto et al., 1974; Bachtarzi and Benmilond, 1983; Hetzel, 1989). Pregnant women have thyroid glands that cannot respond either to thyrotropin or to chorionic gonadotropin, the increased need for thyroxine is not met so serum TSH levels increase (Mandel et al., 1990) It could also be due to the combined results of iodine-deficiency and thyroid changes which occur during pregnancy (Dlange et al., 1972; Pretell et al., 1974; Stevenson et al., 1974). In the normal pregnant women the transient chorionic gonadotropin-induced elevation of the thyroxine production rate is compensated by the hypothalamic-pituitary-thyroid axis, but in hypothyroid pregnant women the thyroid gland cannot respond to thyrotropin or chorionic gonadotropin so the increased need for thyroxine is not fulfilled, hence the serum TSH level rises (Mandel et al., 1990).

As expected, serum T_4 levels were significantly reduced in women of endemic areas as compared to the subjects of non-endemic areas. The reduced T_4 levels have been well established to prevail in the endemic goitre areas (Delange et al., 1972;

Kochupillai et al., 1973; Patel et al., 1973; Pharoah et al., 1973; Thilly et al., 1974; Chopra et al., 1975; Delange et al., 1976; Woeber, 1991). Iodine deficiency causes depletion of thyroid iodine stores leading to reduced production of T_4 (Hetzel, 1989). A decrease in the blood concentration of T_4 triggers the secretion of increased amounts of pituitary TSH, which increases thyroid activity (Hetzel, 1989; Mandel et al., 1990) and results in hyperplasia of the thyroid gland (Hetzel, 1989).

Administration of iodized oil to the pregnant women of the endemic areas, raised the level of T_4 from low to concentrations which were greater than those observed in non-supplemented women of endemic areas. However, T_4 levels in iodized-treated women still remained lower than the T_4 levels observed in women from non-endemic areas during pregnancy and after delivery. These findings are similar to previous observations of Silva and Silva (1981) who studied the effects of oral iodine supplementation (OIS) on thyroid hormones in pregnant women and noted that OIS significantly increased serum T_4 concentrations. Similarly, a number of other studies have also demonstrated that oral administration of iodized oil increases the secretion of serum T_4 (Butfield and Hetzel, 1967; Eltom et al., 1985). Iodine supplementation improves the thyroid function in subjects with or without goitre. By provision of adequate supply of iodine the synthesis of thyroid hormone in the regressed thyroid tissue increases (Goslings et al., 1977).

Iodized oil supplementation also resulted into reduction of TSH from higher concentration in endemic-areas to lower levels in the treated women. With this reduction, concentration of TSH in iodized oil given women became indistinguishable from concentration observed in women of non-endemic areas. This finding is in line with the observations of other workers (Pretell et al., 1969; Silva and Silva, 1981; Abdel-Wahab et al., 1984; Eltom et al., 1985). The reason for suppression of TSH secretion by iodized oil treatment appears to be the elevation of

T_4 which is induced by the treatment and which by negative feed back inhibits TSH secretion. These results clearly indicate that iodine supplementation can correct iodine deficiency by improving serum T_4 levels and decreasing serum TSH concentrations and also reduces goitre size as well.

There was no increase in the serum T_3 levels in the women supplemented with iodized oil. Iodine supplementation did not affect the serum T_3 levels as it did the T_4. Several other workers have also observed that iodine supplementation does not alter the secretion of T_3 (Harada et al., 1979; Silva and Silva, 1981). This indicates that iodine did not induce a change in the capacity of the tissues to convert T_4 to T_3 (Kaplan and Utiger, 1978).

It was interesting to note the reduction of goitre size due to iodized oil supplementation in the women of group 3. Similar results of iodine supplemenation have long been observed by a number of workers (Marine and Kimbal, 1917; Kimbal and Marine, 1918; Kimbal et al., 1919; Marine and Kimbal, 1920). As iodine supplementation causes reduction of the circulating TSH levels, the thyroid gland decreases in size. It has already been shown that iodine supplementation using an oil preparation leads to both a significant decrease of elevated serum TSH levels and an increase in T_4 concentrations in children and in young women (Wachter et al., 1985). Iodine administration in the form of iodized salt, iodized bread or iodized oil was demonstrated to be effective in increasing circulating T_4 and in preventing goitre in adults also (Silva and Silva, 1981; Eltom et al., 1985; Wachter et al., 1985) Iodine administration may also reduce existing goitre in adults (Hetzel et al., 1987). Eltom et al. (1985) observed the effectiveness of oral iodine supplementation in the treatment of endemic goitre and demonstrated that single oral dose of iodized oil can correct iodine deficiency with a reduction in the goitre size and preventing its recurrence for a period of two years. The reduction in goitre size appears to be

secondary to decrease in circulating TSH concentrations which is entrained by administration of the iodized oil (Bautista et al., 1982; Hetzel et al., 1987; Tonglet et al., 1992).

In the present study cord-blood serum T_4 levels were lower and TSH levels were high in the neonates of endemic areas than in neonates of non-endemic areas, with a trend to correcting hormonal levels with the administration of iodized oil in mothers during the pregnancy. In the endemic areas the cord serum T_4 and TSH levels showed a hypothyroid range but no clinical evidence of hypothyroidism was observed in the neonates of group 2. Similar results have been reported by other workers in humans ((Thilly et al., 1974; Sulovic et al., 1984; Kochupillai et al., 1986; Gaitan et al., 1989) and animals (Bachrach et al., 1983). The demonstration of very low cord-blood T_4 levels with increased TSH levels in group 2 is diagnostic of hypothyroidism in the newborns in the goitre-endemic areas and illustrates extent of intrauterine thyroid failure due to iodine deficiency (Kochupillai et al., 1984).

Supplementation with iodized oil significantly raised T_4 levels in the cord blood of infants as compared to the values in infants from non-supplemented mothers of endemic areas. Our findings also indicated that the prevalence of elevated TSH levels in the cord blood of new-borns was decreased when iodine was given to the mothers during early pregnancy. The raised T_4 levels in cord serum of neonates of iodine supplemented mothers were probably due to the consequence of the change in maternal and fetal plasma iodine, as thyroid hormones cannot cross the placenta (Fisher et al., 1977). The thyroid of the new-born from goitre endemic area is in a critical situation as it is competing directly with the maternal thyroid which is greatly in neeed of iodine to meet its own iodine demand as well as that of the fetus (Stanbury, 1972; Sava et al., 1984). Results of the present work are in line with the previous findings of Thilly et al. (1980) and Fisher et al. (1977). However, inspite of

a significant correction of cord-blood T_4 levels by iodized oil supplementation, the T_4 concentrations remained lower than concentration observed in the non-endemic areas.

The present data indicate the persistence of severe goitre in women of endemic areas of Pakistan (groups 2 and 3), which is due to the lack of systematic iodine prophylactic program. In endemic area the prevalence of hypothyroidism was demonstrated from elevated serum TSH levels and decreased T_4 levels. The high TSH and low T_4 levels in this study and several others (Delange et al., 1971; Kochupillai et al., 1973; Patel et al., 1973; Chopra et al., 1975) indicate that thyroid seems to work at the maximum of a limited capacity under maximum endogenous secretion of TSH. Low levels of circulating T_4 are often seen in goitrous subjects and bigger the goitre, the lower are the levels. The hormonal levels can be restored to normal by iodinated salt or iodized oil administration (Eltom et al., 1985; Hintze et al., 1985; Hetzel et al., 1987; Gaitan et al., 1989; Hintze et al., 1989).

Cord-blood serum TSH levels were observed to be high as compared to the values in mothers in all the study groups. In previous studies plasma TSH values were also found to be significantly elevated in cord blood than in maternal blood (Chopra, 1972; Erenberg et al., 1974; Pretell et al., 1974; Walfish, 1976; Khin et al., 1994). After delivery, the serum TSH concentration in the neonates increases rapidly to a peak at 30 minutes of extrauterine life, returning to its initial value within 48 hours (Feely, 1979; Mungan et al., 1994). This neonatal surge of TSH is thought to be due in part to the cooling that follows emergence into the extrauterine environment. This increase also appears to be related to increased thyroptropin releasing hormone (TRH) concentration at the time of birth (Fisher and Klein, 1981). Human placenta is permeable to TRH and there is evidence to suggest that it is also capable of TRH

synthesis (Anderson and Polk, 1990). This extra hypothalamic TRH production thus leads to high levels of TRH in the neonates.

In the current study there was a significant increase in the birth weight of the neonates whose mothers were given iodized-oil in the 1st trimester of pregnancy (group 3) as compared to those of neonates from non-treated mothers of goitre-endemic areas (group 2). However, there was no significant difference in the birth weight of the neonates of group 3 when compared with the group 1 of the non-endemic areas. The previous studies have also shown reduced birth weights in the condition of iodine deficiency in sheep (Hetzel et al., 1983) and monkeys (Hetzel, 1987). Thilly et al. (1980) conducted a trial in an iodine deficient area in Zaire on pregnant women. Iodized oil was administered to the women at 28th week of pregnancy, which resulted in a significant increase in the mean birth weights of the neonates when compared with the controls. This increase in birth weight of neonates in group 3 was probably due to the effects of iodine supplementation to the mothers, which also led to enhanced T_4 secretion in their neonates. This increased production of T_4 may have entrained improved body growth during the fetal development (Fisher, 1985).

The present study indicates that the incidence of spontaneous abortions and stillbirths in the women belonging to the iodine deficient areas was more than in the women of the non-goitre endemic areas which is a frequent and substantial evidence of iodine deficiency and has been reported by a number of workers (Pharoah et al., 1976; Potter et al., 1979; Vojvodic et al., 1993; Giacomucci et al., 1994). Experimental studies conducted on animals also provide consistent evidence of the effect of iodine deficiency on fetal survival (McMichael et al., 1980; Thilly, 1981). The importance of the state of thyroid function in the neonates relates to the fact that thyroid

hormone, dependent on an adequate supply of iodine, is essential for normal brain development.

The incidence of stillbirths, abortions and congenital abnormalities, can be reduced by iodine supplementation during pregnancy (McMichael et al., 1980; Thilly, 1981). Previously, it has been observed that iodine supplementation of iodine-deficient pregnant women also resulted in a significant reduction in fetal and neonatal deaths (Pharoah, 1993). In line with the foregoing observations no incidence of abortion was noticed in the iodine-supplemented women of group 3. Jones and Man (1969) observed that in hypothyroid patients the rate of spontaneous abortion was high as compared to the euthyroid pregnant women. Other studies have also provided evidence that thyroxine replacement therapy reduces early pregnancy outcome (Jones and Man, 1969; Greenman et al., 1962; Niswander and Gordon, 1972; Winikoff and Malinek, 1975).

The number of abnormal children observed in the families of women included in the study was higher in the goitre-endemic areas as compared to the women of non-endemic areas. The typical signs observed were retarded physical growth and squint eyes. Some growth retardation could be due to poor nutritional status but it may also be a feature of iodine deficiency (Hetzel and Hay, 1979). Squint eyes are often found in association with iodine deficiency and have been observed by many other workers (Hetzel 1983; Anonymous, 1988). In addition to these children two neurological cretins were present in the families of group 3 and one was born during the study period in the group 2. The clinical features of the cretin born were similar to those of the neurological cretins i.e. mental retardation, deaf-mutism and spastic paralysis found in other areas of the world (Dodge et al., 1969; Ma et al., 1986).

The low hemoglobin levels observed in the pregnant women of different groups in the present study indicate the presence of iron deficiency anemia. These findings are in line with previous observations (Anonymous, 1977; Anonymous 1988; Karim et al., 1994). Iron deficiency anemia in the women of different study groups which were of low socio-economic status can be attributed to the poor bioavailability of the iron in the diet, which is mostly contributed by wheat flour, and the presence of inhibitors of iron absorption in the diet, such as phytates and tannins.

The present study is the first of its kind that assesses clinical and endocrine status of maternal and neonatal thyroid from both goitre-endemic and non-goitre endemic areas in Pakistan. These investigations have demonstrated that iodine deficiency in the areas studied, leads to hypothyroidism as indicated by reduction in thyroid hormones, increased reproductive losses, a reduction in birth-weight and an increased incidence of abnormal children, possibly reflecting reduced mental function. In communities suffering from iodine deficiency, there may be reduced capacities for initiative and decision making leading to socio-economic retardation in addition to other health problems. Present data also clearly establishes that iodine supplementation during pregnancy can reverse hypothyroidism in mothers and their babies and would also lead to increased body growth of neonates.

Therefore, present findings warrant the induction of iodine prophylactic policies in the goitre-endemic areas and emphasize the need for comprehensive thyroid screening program of neonates. These measures will help in eliminating the iodine deficiency disorders and hence would contribute to improved life quality in the iodine deficient areas of Pakistan.

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Annexure 1

Explanation

I being the student of the Department of Biological Sciences of Quaid-i-Azam University Islamabad is conducting a research study on endocrine and clinical assessment of thyroidal function in pregnant mothers and neonates. This will help in finding ways to improve the health status of women and children. Your cooperation will help in improving the status and quality of life in your area. To perform the study we need administration of iodized oil to you and colleciton of your blood samples.

Consent:

I _____ Age _____ resident of _____ have been told about the Research Project details. I am willing to participate in this study.

Signature / Thumb Impression:

Address _____

_____

_____

Witness

Signature _____

Name _____

Address _____

_____

Annexure 2

Proforma

Code _____

Village/ Teh _____

House Identification _____

Name of Subject _____ Age _____

Husband Name _____ Occupation _____

Number of children Living _____ Died _____

Number of Pregnancies _____ Abortions _____

Still Births _____ Miscarriages _____

Abnormal child Yes _____ No _____

Symptoms _____

Iodine supplementation Yes _____ No _____

Stages of Goitre 0 _____ 1 _____ 2 _____

Reduction in Goitre size _____ _____ _____

Height (cm) _____

Weight (Kg) _____

Hb (gm/100 ml) _____

Blood samples collected trimester wise 1st _____ 2nd _____ 3rd _____

Post partum _____

Pregnancy outcome Live _____ Dead _____ Others _____

Sex of the child born Male _____ Female ____

Birth Weight (Kg) _____

Clinical examination _____

_____

Cord blood sample collected Yes _____ No _____

Annexure 3

T_3 Enzyme Immunoassay Procedure

1. Pipette 0.1 ml of Zero Standard into the duplicate zero tubes and 0.1 ml of the remaining standards, samples and control into the labeled tubes in duplicate.
2. Dispense 0.1 ml of Serozyme T_3 Derivative into each tube.
3. Dispense 0.1 ml of Serozyme Anti-T_3 Regent into each tube. Cover the rack with plastic film. Gently vortex-mix all the tubes using a multivortex.
4. Incubate the rack of tubes for 15 minutes in a 37°C waterbath.
5. Dispense 0.2 ml of thoroughly mixed Separation Reagent into each tube, including the Zero Standard tubes. Cover the tubes and gently vortex-mix the rack.
6. Incubate the rack of tubes for 10 minutes in a 37 °C waterbath.
7. Slide the rack of tubes into the Magnetic Separator for 2 minutes.
8. Decant the supernatant from all tubes in the rack by inverting the Separator in one large, slow circular movement. Place the inverted Separator on absorbent paper and hit the base to dislodge any droplets of liquid adhering to the sides of the tubes.
9. Set the Separator upright and add 0.5 ml of diluted Wash Buffer to each tube.
10. Remove the rack from the Separator and place it on the multi-vortex mixer.
11. Slide the rack of tubes into the Magnetic Separator and wait for 2 minutes.
12. Repeat step 8
13. Label one or two tubes for blanking the Photometer and place them in the rack.
14. Remove the rack from the Separator and pipette 0.3 ml of Serozyme Substrate Solution into each tube, along the blanks. Cover the rack and Vortex-mix all the tubes.
15. Incubate the rack for 15 minutes in a 37 °C waterbath.
16. Pipette 1.0 ml of Serozyme Stop Solution into each tube, including blank tubes.
17. Slide the rack into the Magnetic Separator for at least 10 minutes.
18. Blank Serono Photometer at 550 nm and measure the absorbances for standards, controls and serum samples at the same wavelength and at 492 nm.

Annexure 4

T_4 Enzyme Immonoassay Procedure

1. Pipette 0.05 ml of Zero Standard into the duplicate zero tubes and 0.05 ml of the remaining Standards, samples and control into the labeled tubes in duplicate.
2. Dispense 0.2 ml of Serozyme T_4 Derivative into each tube.
3. Dispense 0.2 ml of Serozyme Anti-T_4 Reagent into each tube. Cover the rack with plastic film and gently vortex-mix all the tubes.
4. Incubate the rack of tubes for 15 minutes in a 37°C waterbath.
5. Dispense 0.2 ml of thoroughly mixed Separation Reagent into each tube, including the Zero Standard tubes. Cover the rack and vortex-mix all tubes.
6. Incubate the rack of tubes for 10 minutes in a 37°C waterbath.
7. Slide the rack of tubes into the Magnetic Separator for 2 minutes.
8. Decant the supernatant from all tubes in the rack by inverting the Separator in one large, slow circular movement. Place the inverted Separator on absorbent paper and hit the base firmly to dislodge any droplets of liquid adhering to sides of the tubes.
9. Set the Separator upright and add 0.5 ml of diluted Wash Buffer to each tube.
10. Remove the rack from the Separator. Vortex vigorously on multi-vortex mixer.
11. Slide the rack of tubes into the Magnetic Separator. Wait for 2 minutes.
12. Repeat step 8.
13. Label one or two tubes for blanking the Photometer and place them in the rack.
14. Remove the rack from the Separator and pipette 0.3 ml of Serozyme Substrate Solution into each tube, along the blank tubes. Cover the rack, Vortex-mix all the tubes.
15. Incubate the rack for 15 minutes in a 37°C waterbath.
16. Pipette 1.0 ml of Serozyme Stop Solution into each tube, including blank tubes.
17. Slide the rack into the Magnetic Separator for at least 10 minutes.
18. Blank Photometer at 550 nm using blank tubes and measure the absorbances for standards, controls and serum samples at the same wavelength and at 492 nm.

Annexure 5

TSH Immunoenzymetric Assay Procedure

1. Pipette 0.2 ml of Zero Standard into the duplicate zero tubes and 0.2 ml of the remaining Standards, samples and controls into the labeled tubes in duplicate.
2. Dispense 0.2 ml of Serozyme Anti-TSH Reagent into each tube. Cover the rack and gently vortex-mix all the tubes using a multi-vortex mixer.
3. Incubate the rack of tubes for 30 minutes in a 37°C waterbath.
4. Dispense 0.2 ml of thoroughly mixed Separation Reagent into each tube, including the Zero Standard tubes. Cover the rack and gently vortex-mix all the tubes.
5. Incubate the rack of tubes for 5 minutes in a 37°C waterbath.
6. Slide the rack of tubes into the Magnetic Separtor for 2 minutes.
7. Decant the supernatant from all tubes in the rack by inverting the Separator in one large, slow circular movement. Place the inverted Separator on absorbent paper and hit the base firmly to dislodge any droplets of liquid adhering to the sides of the tubes.
8. Set the Separator upright and add 0.5 ml of diluted Wash Buffer to each tube.
9. Remove the rack from the Separator & Vortex vigorously on multi-vortex mixer.
10. Slide the rack of tubes into the Magnetic Separator. for 2 minutes.
11. Repeat step 7.
12. Repeat steps 8-11.
13. Label one or two tubes for blanking the Photometer and place them in the rack.
14. Remove the rack from the Separator and pipette 0.3 ml of Serozyme Substrate Solution into each tube, along the blanks. Cover the rack and Vortex-mix all the tubes.
15. Incubate the rack for 60 minutes in a 37°C waterbath.
16. Pipette 1.0 ml of Serozyme Stop Solution into each tube, including blank tubes.
17. Slide the rack into the Magnetic Separator for at least 10 minutes.
18. Blank Photometer at 550 nm using the blank tubes and measure the absorbances for standards, controls and serum samples at the same wavelength and at 492 nm.