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Impact Of Drinking Water, Dietary Habits And Environmental Factors On Thyroid Hormones

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ABSTRACT
Back ground: Thyroid disorders affect millions globally, involving complex interaction between genetic and environmental factors. Recent studies indicated that thyroid disorders are prevalent in the hilly regions, Karak district, Khyber Pakhtunkhwa province in Pakistan had been overlooked due to extreme conservatism present in the region.
Objectives: The objectives of the study were to determine the frequency and distribution of thyroid disorders among the female population residing in Karak district and to find out if factors like ground water and the local edibles contribute the development of thyroid disorders or thyroid gland status in studied area.
Methods: The consumable water were collected from four scattered sources (hand pumps, tap water, wells and springs) in each locality of the study area (Zebichenakhel, Lawagharchenakhel and Upper Lawagharchenakhel).The water samples were processed for the characteristics of pH and conductivity and for the concentrations of total dissolved solids, iodine, fluoride, selenium, nitrate and cyanide using specific spectrophotometric methods. These were compared with WHO permissible ranges to assess in general the quality of the water consumed by the studied population.
Results: The occurrence of thyroid disorders was 33%(100)and 14% were normal euthyroid in these areas conformed through ELISA kits. The foods grown in the area of the district Karak and consume by the female population was focused and the information was collected from each of the subject sampled for the blood and whose thyroid function's hormones profile was investigated. The food component contain cruciferous vegetables whose high intake can cause hypothyroidism because these vegetables naturally contain substances goitrogens interfering in thyroid hormones synthesizing capacity. The hypothyroid incidences may be iodine induced due to uneven that is some taking and other avoiding iodine supplementation. To further understand these elements and how they might assist individuals in diagnosing and treating thyroid diseases, more research is necessary.

Key Words: Thyroid hormone, TDS, Iodine, Floride

Introduction:
Environment, dietary habits, and physiological adaptations in the diversified human population bring a specific pattern of normal and thyroid disorders. Global incidence of thyroid diseases is from 5% to 10% (Strikic *et al.,* 2022). Thyroid dysfunction is a widespread endocrine disorder worldwide, with varying prevalence rates across countries. India has a higher prevalence of 11%, while the UK and US have lower rates of 4.6% and 2%, respectively. Factors such as genetic predisposition, dietary habits, environmental factors, and healthcare access influence these variations (Das *et al.,* 2022, Strikic *et al.,* 2022 and Mousa, & Zoori, 2023).
Groundwater is considered as a vital water source in many parts globally due to its economic and health benefits. Unfortunately, approximately 80% of health issues and diseases worldwide can be attributed to the consumption of contaminated water for domestic purposes (Panneerselvam *et al.*, 2023). The micronutrients in ground water play a crucial role in the growth and reproduction of plants, animals, and humans. Essential micronutrients humans are Cu, Fe, Mn, Mo, Co, I, Se, F, and Cr (Shukla *et al.*, 2018). Among all nutrients, a micronutrient called selenium is found in many proteins. The organ in humans with the most selenium per gram of tissue is the thyroid (Gorini *et al.*, 2021).The amount of selenium in a person's body is influenced by their nutrition, environment, geographic location, and soil composition. Selenium is necessary for thyroid hormone metabolism and antioxidant activity in the thyroid (Venture *et al.*, 2017).

Nitrates in water or foods inhibit thyroid iodide uptake by binding to the sodium-iodide symporter, affecting thyroid hormone synthesis and release. This leads to a negative feedback response, causing elevated levels of thyroid stimulating hormone, causing a hypothyroid-like state (Kherad *et al*., 2016). Elevated TSH stimulation in nitrates leads to hypertrophic effects on the thyroid, increasing its size and causing sub-clinical hypothyroidism (Zeng *et al*., 2012). Kheradpisheh *et al*., (2018) mentioned the impact of drinking water fluoride (F^-) on human thyroid hormones. The effects of high fluoride intake from consumable water, through large consumption of green tea, and other reasons on thyroid hormones and TSH have been studied in both humans and animals. Several studies have reported a lowering of the T4 and T3 accompanying marked increase in the TSH levels (Zeng *et al*., 2012). In a northern Mexican study the fluoride as low as 1 mg/L reduced T3 levels in teenagers inhabiting the region (Ruiz-Payan *et al*., 2012). Fluoride-contaminated water was discovered to cause thyroid gland problems, particularly in pregnant women, where abnormal hypothyroidism raises blood pressure during late pregnancy, increasing the chance of pregnancy before the 20th week (miscarriage) (Kheradpisheh *et al*., 2018).
Cyanide concentrations in consumable water sources are generally quite low. Historical data collected from the late 1970s to early 1980s across the United States by the United States Environmental Protection Agency's database reported levels below 3.5 μg/l in most areas. However, some limited areas showed higher levels, occasionally exceeding 200 μg/l (ATSDR, 1997). Research on rats revealed that cyanide and its derivative thiocyanate could reduce thyroid gland activity. This effect was more pronounced in rats on restricted diets, leading to decreased thyroxin levels in circulation and enlarged thyroid glands due to elevated TSH feedback mechanisms (Philbrick *et al*., 1979). Substances such as cyanide, nitrate, and flavonoids can potentially affect hormones and the human body, especially the thyroid gland, thereby impacting overall health (Umar *et al*., 2023).
Despite the availability of the iodine from the environmental sources being sufficient in consumable water and the foods a condition like of iodine deficiency for the thyroid ultimate functions of production of thyroid hormones are created by the chemicals in consumable water and the food sources. Several nutritional factors of goitrogens refer to a collection of micronutrients that can cause broadening of the thyroid gland (Babiker *et al.,* 2020).
The Brassicaceae family of plants includes cruciferous vegetables including kale, turnips, cauliflower, and others that are high in indole glucosinolate. This substance was discovered to break down in animal tests into the goitrin metabolite thiocyanate, which prevents the thyroid cell from absorbing iodine. It functions by limiting thyroid hormone production by competitively blocking the sodium/iodide symporter (Felker *et al*., 2016).
The cruciferous vegetables like broccoli, cauliflower, and cabbage naturally release a substance called goitrin. It's possible for goitrin to prevent thyroid hormones from being made. However, unless there is also an iodine deficit, this typically isn't a problem according to the Institute of Medicine, Food and Nutrition Board, 2001. Heat denatures much or all of this putative goitrogenic impact in cruciferous vegetables (Fuller *et al*., 2007). Cruciferous vegetables, were also attributed to cause thyroid dysfunction. Environmental factors, namely, contamination of water with goitrogens could also contribute to the etiology of goiter in some endemic area (Babiker *et al*., 2020).

Materials and Methods:
The samples were collected from the participants residing in Karak District (Zebichenakhel, Lawagharchenakhel and Upper Lawagharchenakhel).These areas exists in the north-western part of Pakistan and contains the southern stretches of the Hindu Kush range of Himalayan Mountains. It is a comparatively low mountains' area of Khyber Pakhtunkhwa (KPK).

Blood sampling:
The blood samples were collected from the female population randomly at the location were analyzed through competitive Enzyme Immunoassay method (ELISA) by using the kits for biochemical estimation of (Thyrotropin (TSH), Total Thyroxine (TT4), Free Thyroxin (FT4) Total Triidothyronin (TT3) and Free triidothyronin (FT3) (Maqbool *et al.,* 2019, Mansoor *et al.,* 2022, Gar-Elnab *et al*., 2013).The kits which were used to process the blood samples were AccuBind ELISA Microwells of Monobind Inc, USA.

Consumable water sampling Samples of the consumable water were collected from four scattered sources (hand pumps, tap water, wells and springs) in each locality of the study area (Zebichenakhel, Lawagharchenakhel and Upper Lawagharchenakhel). Samples were collected in polyethylene (PE) bottles obtained from the laboratory (Global Environmental Lab, Lahore) for the analysis of the water where it had been done.
The water samples were processed for the characteristics of pH and conductivity and for the concentrations of total dissolved solids, iodine, fluoride, selenium, nitrate and cyanide. These were compared with WHO permissible ranges to assess in general the quality of the water consumed by the studied population.

Water Samples Analysis: The water samples were processed for the characteristics of pH using pH Mater EUTECH pH-700 by APHA 4500 H^+ Electrometric Method (Bates, 1978) and conductivity by EC/TDS/NaCl Meter (Mekonnen *et al*., 2020). Total Dissolved Solids in the samples was determined using method APHA 2540 C. The concentrations of, iodine in the samples was determined using method Spectrophotometer DR 2800 HACH USA by DPD Fluoride of samples was determined using method HACH 8029/ APHA 4500-F^-D SPANDS by Spectrophotometer DR 2800 HACH USA (Somasundaram *et al*., 2015Selenium of samples was determined using method APHA 3113 B Electro-thermal Atomic Absorption Spectrophotometric (Vučković *et al*., 2016). Nitrate of samples was determined using method HACH 8039 by Spectrophotometer DR 2800 HACH USA (Kazi *et al*., 2017). Cyanide of samples was determined using HACH 8027/ APHA 4500-CN^- E Colorimetric Method by Spectrophotometer DR 2800 HACH USA (Chakraborty and Bhattacharya, 2017).

Food: The food material grown in the area of the district Karak and consume by the female population was focused and the information was collected from each of the subject sampled for the blood and whose thyroid function's hormones profile was investigated. The vegetables grown with available water in these areas are listed out as; mustard greens, broccoli, cauliflower, cabbage, spinach, spring onion, brinjal, leafy greens and pees. Each sampled subject was interviewed, and the information collected by the researcher as most of the subjects were illiterate. The list of the consumable vegetation (mustard greens, broccoli, cauliflower, cabbage, spring onion, brinjal, leafy greens and pees) was obtained along the frequency of the consumption around in a week. In this way an overall view was possible to present the frequency in numerical strength of 1, 1+, 2, 2+, 3 and 3+ for a particular vegetation.

Statistical analysis: The data was of parametric type and parametric statistical tests were applied. Firstly, descriptive statistics (mean, number, standard error of mean) was performed for the subjects in the groups. Percentage comparisons were calculated in order to measure the magnitude of the difference between the groups. The comparison between the two groups was conducted using the unpaired student t test. One-way analysis of variance (ANOVA) was used to relate the means of more than two groups, all those parametric tests were applied by using a software named Minitab 'Statistical Package version 17'. The level of statistical significance was analyzed by value of probability (p-value) in those statistical tests, <0.05 p-value represented significant difference between the groups (Minitab 'Statistical Package version 17).

RESULTS:

In the present study, three localities were chosen in Karak district which included Zebichenakhel, Lawagharchenakhel and Upper Lawagharchenakhel to assess the thyroid disorder status in randomly selected female subjects with 15 to 70 years age from each locality.

Distribution of Thyroid disorders in different areas of Karak district: Table 1 illustrates the occurrence of thyroid and associated abnormalties in studied areas of Karak district. Highest percentage (46.1%) of euthyroid were present in Lawagharchenakhel and 33.3% were present in the Upper Lawagharchenakhel. Furthermore the euthyroid status percentage in Zebichenakhel was 18%.Additionally, the highest number of hypothyroid subjects comprising 53% were found in the Zebichenakhel. Furthermore the percentage of hypothyroid status was 30.76% in Lawagharchenakhel and The lowest percentage of 13% present in Upper Lawagharchenakhel**.** Furthermore, the percentage of hyperthyroid status was 13% in Upper Lawagharchenakhel, 7.69% in Lawagharchenakhel. The lowermost number of hyperthyroid subjects were observed with the percentage of 6% present in Zebichenakhel.

Table 1: Thyroid and associated abnormalities in the studied area

S.No	**Area**	**Euthyroid**	**Thyroid abnormalities**	
			Hypothyroid	**Hyperthyroid**
1	Zebichenakhel	3(18%)	9(53%)	1(6%)
2	Lawagharchenakhel	6(46%)	4(30%)	1(7%)
3	Upper Lawagharchenakhel	5(33%)	2(13%)	2(13%)
Total %age		14(42.4%)	15(45%)	4(12%)

Water samples analysis: The primary motivation for testing the water quality was due to the fact that the subject population used ground well water for drinking and other reasons like local vegetation. The pH, conductivity, total dissolved solids, iodine, fluoride, selenium, nitrate, and cyanide contents of the water samples were also measured using various methods to evaluate the general water quality values of the investigated community, these were observed values compared to WHO-permitted ranges (WHO, 2011).

pH: The pH status of the collected samples is presented in Fig.1 a. Tab 2a.The analyzed water sample ranged between 7.9 to 8.2 in all the samples collected from the study sites. The mean pH among the different localities ranged between 7.9250 ± 0.0479 to 8.2750 ± 0.0479. This demonstrated the pH of all the localities on the alkaline side. The pH of Zebichenakhel was significantly more alkaline compare to other localities. WHO permissible range for consumable water: pH 6.5 to 8.5 (WHO, 2011).

Conductivity: Fig.1 b. Tab 2 b. Indicates the conductivity range of water samples from each locality. The water samples showed the conductivity in the range of 710 to 775 uSiemens/meter in all the samples collected from the sites. The mean conductivity at the different localities of the studies ranged between 719.75 ± 4.48 and 758.26 ± 4.90 uSiemens/meter. The conductivity of Upper Lawagharchenakhel was noted significantly lower than the rest of the localities.

Total dissolved solids: The water samples showed the contents of total dissolved solids in the range of 337ppm to 420 ppm in all the samples collected from the sites. The mean conductivity at the different localities of the studies ranged between 373 ± 8ppm and 399.75 ± 3.91ppm. The contents of total dissolved solids did not exhibit any significant difference from one another (Fig.1 c.Tab 2 c).

Table 2 a. Mean pH of water samples from different sources of each of the locality.

Locality	Hand pumps	Tap water	wells	Springs	Mean ± SEM
Zebichenakhel	8.2	8.4	8.3	8.2	8.2750 ± 0.0479
Lawagharchenakhel	8.0	7.9	7.9	8.0	7.9500 ± 0.0289
Upper Lawagharchenakhel	7.8	8.0	8.0	7.9	7.9250 ± 0.0479

WHO permissible range for consumable water: pH 6.5 to 8.5 (WHO, 2011).

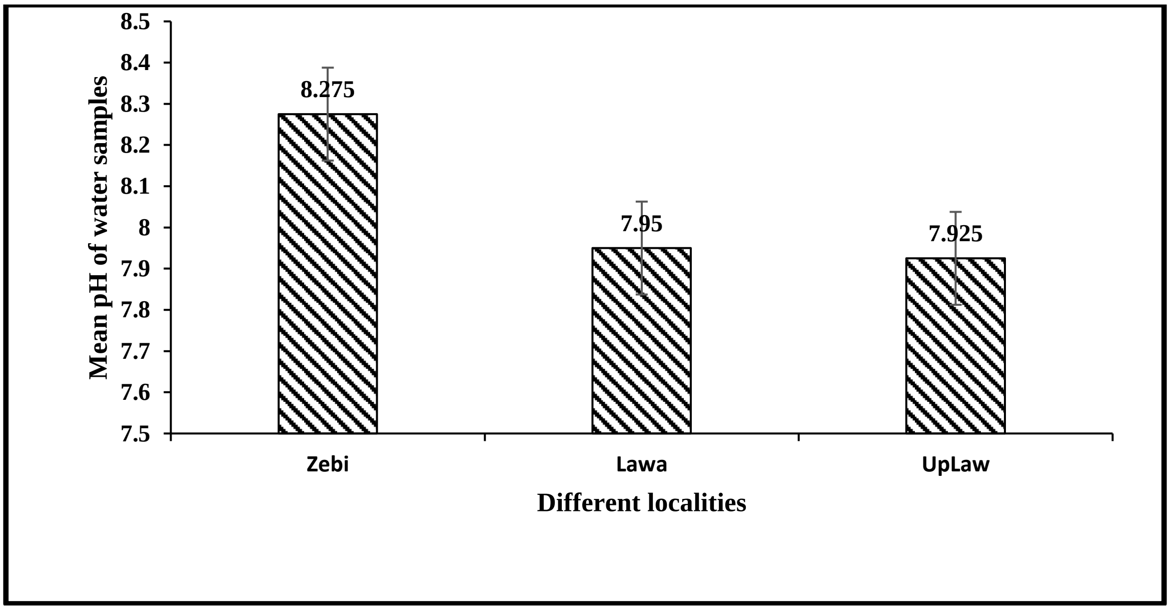

Figure 1a. Mean pH of water samples from different sources of localities such as Zebi(Zebichenakhel),Lawaghar(Lawagharchenakhel),Uplaw(Upperlawagharchankhel).Significantly different P=<0.05. #Lawagharchenakhel, %Upper Lawagharch from *zebi.

Table 2 b: Conductivity uSiemens/meter of water samples from different sources of each of the locality.

Locality	Hand pumps	Tap water	wells	Springs	Mean ± SEM
Zebichenakhel	758	765	702	770	748.8 ± 15.8
Lawagharchenakhel	761	748	739	775	755.75 ± 7.85
Upper Lawagharchenakhel	710	722	731	716	719.75 ± 4.48

WHO permissible limit: 1000 uSiemens/meter (WHO, 2011).

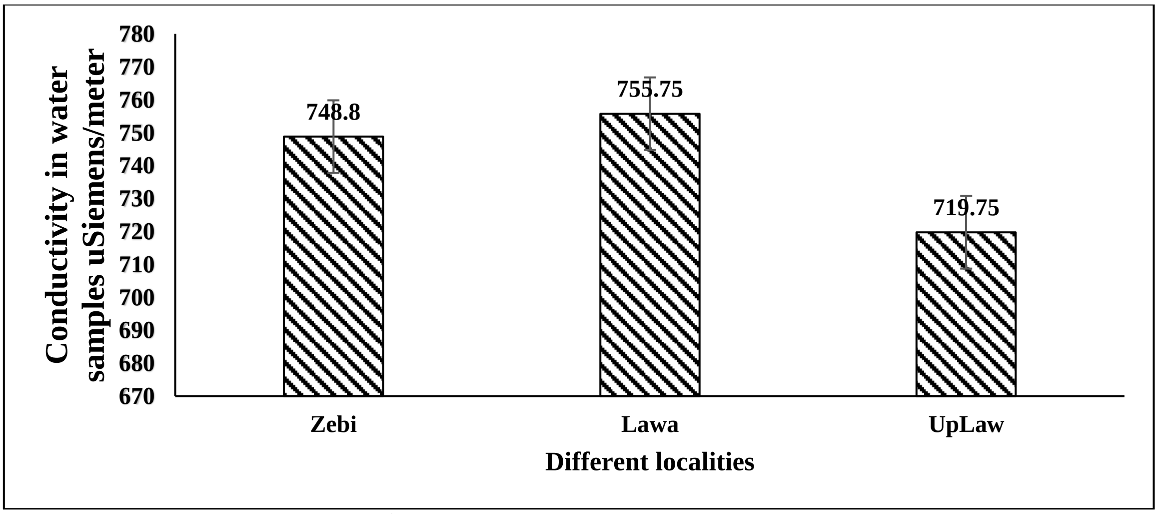

Figure 1 b. Mean Conductivity uSiemens/meter of water samples from different localities such as Zebi (Zebichenakhel), Lawaghar (Lawagharchenakhel), Uplaw (Upper Lawagharchenakhel) was significantly different P=<0.05,% Upper Lawagharchenakhel was significantly different.

Table 2 c. Total Dissolved Solids (TDS) ppm of water samples from several sources of each of the locality

Locality	Total Dissolved Solids of water samples from various resources of the area				Mean ± SEM
Zebichenakhel	384	395	410	402	399.75 ± 6.33
Lawagharchenakhel	337	375	401	382	373.8 ± 13.4
Upper Lawagharchenakhel	391	384	420	395	397.5 ± 7.84

WHO maximum permissible in consumable water: 1000mg/L(WHO,2011)

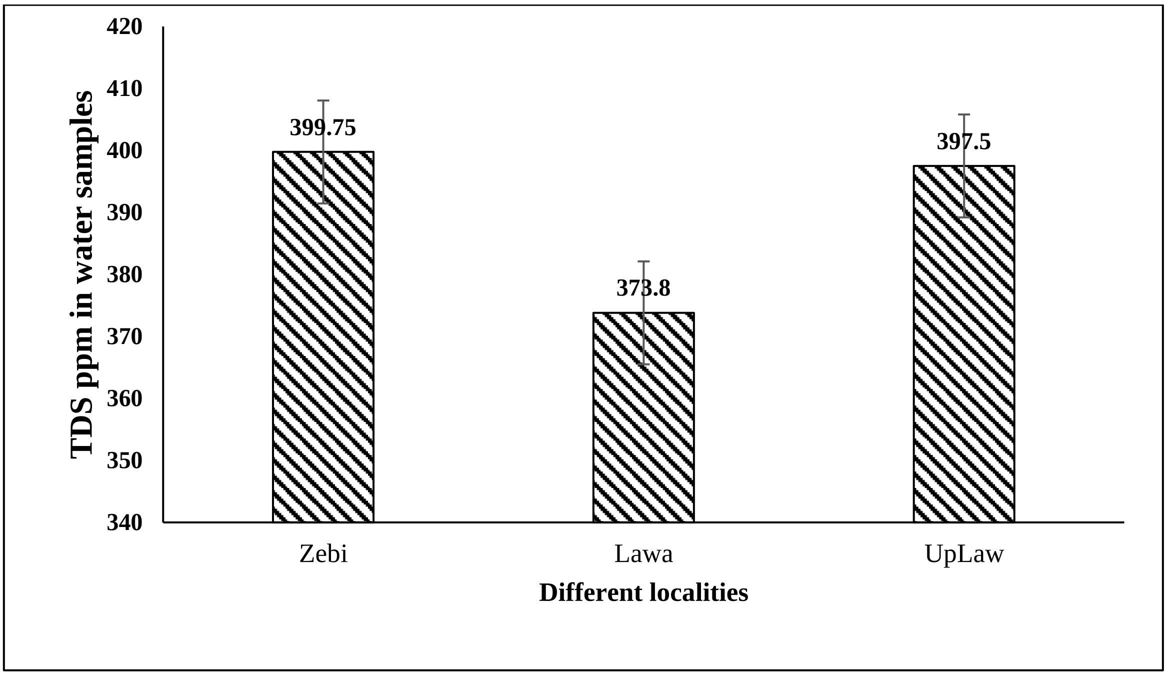

Figure 1 c: Mean content of Total Dissolved Solids (TDS) ppm of water sources from different localities such as Zebi (Zebichenakhel), Lawaghar (Lawagharchenakhel), Uplaw (Upper Lawagharchenakhel). Significantly different P=<0.05

Iodine: The iodine contents of all the water samples at different localities showed the range of 10 to 32ug/L. The mean iodine concentration at the three localities of the studies ranged between 14.25 ± 2.21ug/L and 28.75 ± 0.854ug/L. The iodine concentration was noted the lowest at Zebichenakhel and the maximum at Upper Lawagharchenakhel. At Zebichenakhel iodine contents were highly significantly approximately 50% lower (P=0.000) than the concentration at Upper Lawagharchenakhel. Similarly the iodine lower magnitude than Zebichenakhel. The difference in the iodine contents in water samples of different localities has been observed (Fig.2 a Tab 3 a).

Fluoride: The fluoride contents of all the water samples at different localities showed the range of 0.1 to 0.2mg/L. The mean fluoride concentration at the different localities of the studies ranged between 0.1125 ± 0.0125ug/L and 0.1625 ± 0.0329mg/L. The fluoride concentration has been observed greater at Upper Lawagharchenakhel compare to the others. In general very low level of fluoride was observed in the water samples of the study localities (Fig 2 b Tab3 b).

Selenium: The selenium contents of all the water samples at different localities showed the range of 0.5 to 12.2ug/L. The mean iodine concentration at the different localities of the studies ranged between 2.150 ± 0.870ug/L and 8.07 ± 1.47ug/L. The selenium concentration in the water samples was found significantly lowest at Zebichenakhel and significantly at Upper Lawagharchenakhel compare to the mineral content (Fig.2c Tab.3c) **.**

Nitrate: The concentration of nitrate in all the water samples at different localities exhibited the range of 5 to 18mg/L. The mean nitrate concentration at the different localities of the studies ranged between 5.750 ± 0.479mg/L and 15.25 ± 1.25mg/L. The nitrate concentration in the water samples was found significantly greater at Upper Lawagharchenakhel compare to other localities. It was found lowest at Zebichenakhel and upper Lawagharchenakhel (Fig.2d, Tab 3 d).

Cyanide: The concentration of cyanide in all the water samples at different localities exhibited the range of 8 to 55ug/L. The mean CN concentration at the different localities of the studies ranged between10.5 ± 1.04ug/L and 41.5 ± 2.10g/L. The highest concentration of cyanide was estimated at Zebichenakhel and Lawagharchenakhel. The levels in both of these are highly significant than the rest of the localities. The lowest level of the cyanide was found at Upper Lawagharchenakhel and its level is almost 75% lower than the concentrations of Zebichenakhel and Lawagharchenakhel. (Fig.2 e, Tab 3e).

Table 3 a:Iodine ug/L in water samples from different sources of each of the locality.

Locality	Hand pumps	Tap water	wells	Springs	Mean ±SEM
Zebichenakhel	10	11	17	19	14.25 ± 2.21
Lawagharchenakhel	19	22	21	20	20.50 ± 0.645
Upper Lawagharchenakhel	27	29	31	28	28.75 ± 0.854

Range from several studies: 5 to 30ug/L(WHO, 2011).

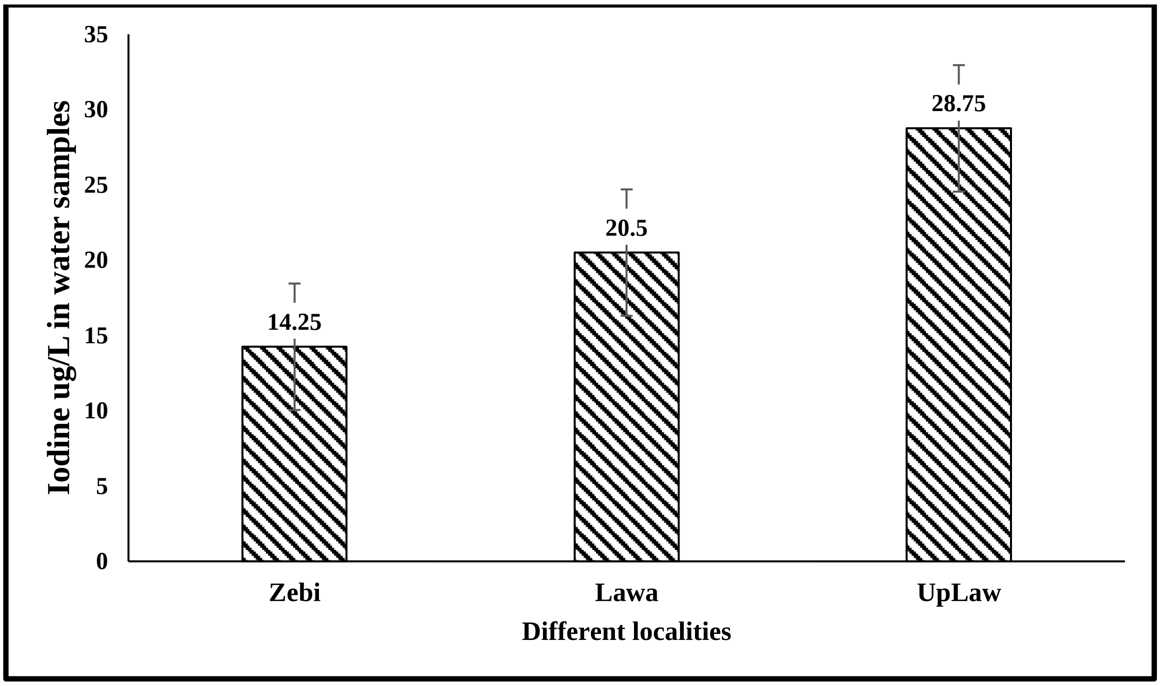

Figure 2 a. Mean iodine ug/L in water collection from various resources of each of the locality such as Zebi (Zebichenakhel),Lawaghar(Lawagharchenakhel),Uplaw(UpperLawagharchenakhel).Significantly different P=<0.05 % Upper Lawagharchenakhel ware significantly different from*Zebichenakhel. %Upper Lawagharchenakhel shows significantly difference.

Table 3 b. Fluoride mg/L in samples of water taken from various sources of localities.

Locality	Hand pumps	Tap water	wells	Springs	Mean ± SEM
Zebichenakhel	0.1	0.1	0.15	0.1	0.1125±0.0125
Lawagharchenakhel	0.1	0.15	0.1	0.1	0.1125±0.0125
Upper Lawagharchenakhel	0.2	0.15	0.2	0.1	0.1625±0.0239

WHO maximum permissible limit in consumable water: 1.5mg/L (WHO, 2006).

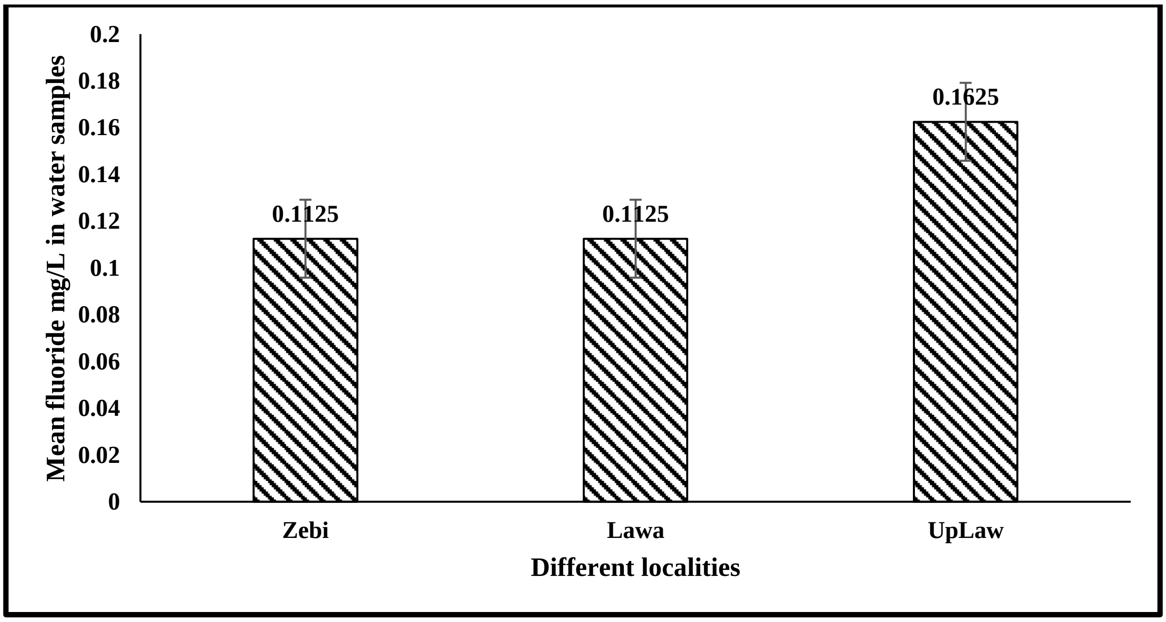

Figure 2 b: Mean fluoride mg/L in water samples from different sources of each of the locality such as Zebi

(Zebichenakhel), Lawaghar (Lawagharchenakhel), Uplaw(Upper Lawagharchenakhel),. Significantly different P=<0.05.

Table 3 c.Selenium ug/L in water samples from different sources of each of the locality.

Locality	Hand pumps	Tap water	wells	Springs	Mean± SEM
Zebichenakhel	0.5	0.8	3.5	3.8	2.150±0.870
Lawagharchenakhel	3.1	4.5	3.9	5.8	4.325±0.569
Upper Lawagharchenakhel	8.2	6.1	12.2	5.8	8.07± 1.47

WHO maximum permissible in consumable water: 40ug/L (WHO, 2003). cantly different P=<0.05.

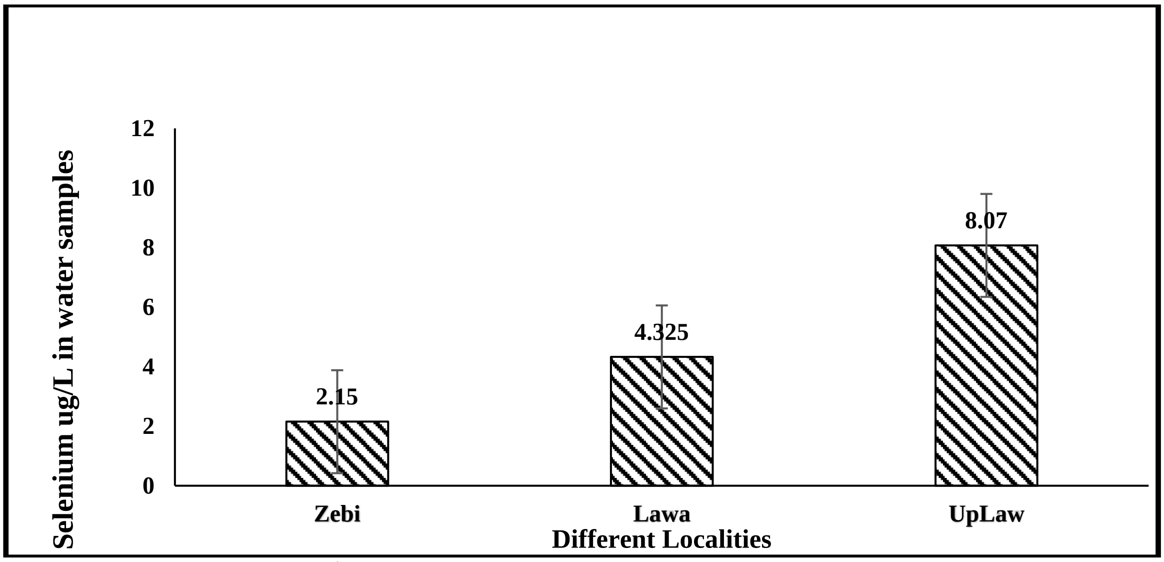

Figure 2 c. Mean selenium ug/L in water samples from diverse resources of each of the locality such as Zebi (Zebichenakhel), Lawaghar (Lawagharchenakhel), Uplaw (Upper Lawagharchenakhel). Significantly different P=<0.05. % Upper Lawagharchenakhel was significantly different from *Zebichenakhel.

Table 3 d. Nitrate mg/L in water samples from different sources of each of the locality.

Locality	Hand pumps	Tap water	wells	Springs	Mean ± SEM
Zebichenakhel	6	8	7	5	6.500 ± 0.645
Lawagharchenakhel	11	10	9	7	9.250 ± 0.854
Upper Lawagharchenakhel	16	18	12	15	15.25 ± 1.25

WHO maximum permissible limit: 50mg/L (WHO, 2003).

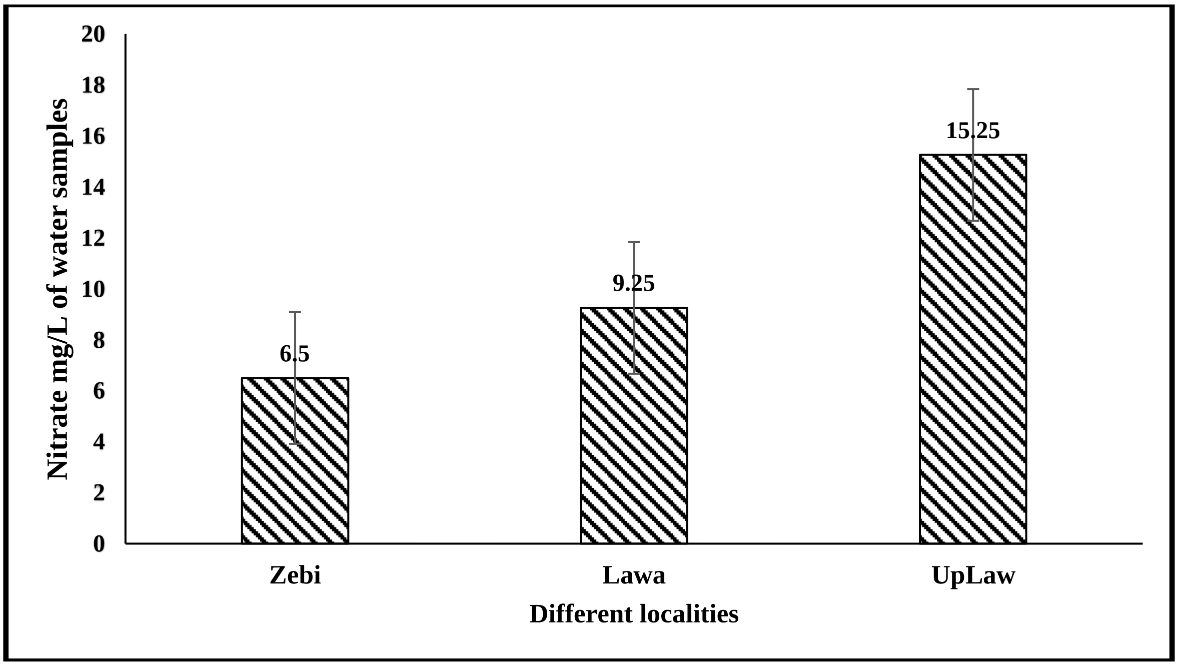

Figure 2 d: Mean nitrate mg/L in water samples from various sources of each of the locality such as Zebi (Zebichenakhel), Lawaghar(Lawagharchenakhel), Uplaw (Upper Lawagharchenakhel). Significantly different P=<0.05.*Zebichenakhel were significantly different from % Upper Lawagharchenakhel, %Upper Lawagharchenakhel, # Lawagharchenakhel shows significantly difference result.

Table 3 e: CN (Cyanide) ug/L in water samples from different sources of each of the locality.

Locality	Hand pumps	Tap water	wells	Springs	Mean ± SEM
Zebichenakhel	55	48	33	29	41.25±6.14
Lawagharchenakhel	46	36	41	43	41.50±2.10
Upper Lawagharchenakhel	10	8	11	13	10.50±1.04

WHO maximum permissible limit: 70ug/L (WHO, 2011).

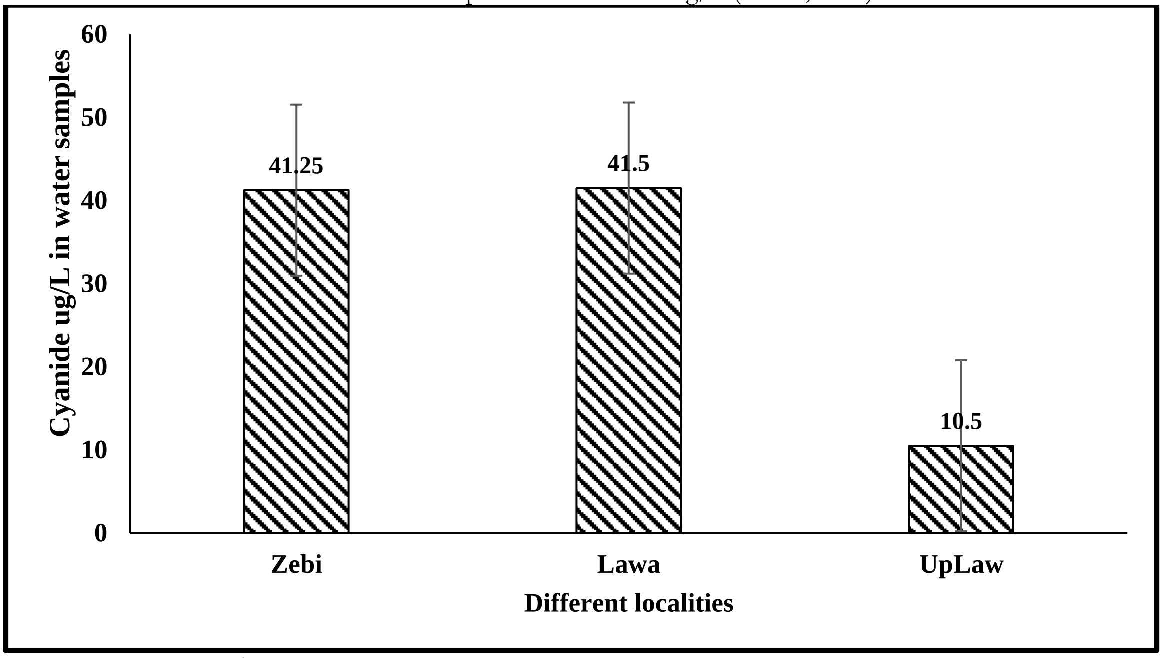

Figure 2 e: CN (Cyanide) ug/L in water samples from different sources of each of the locality such as Zebi (Zebichenakhel), Lawaghar (Lawagharchenakhel), Uplaw (Upper Lawagharchenakhel). Significantly different P=<0.05. %Upper Lawagharchenakhel was significantly different from *Zebichenakhel, % Upper Lawagharchenakhel and # Lawagharchenakhel verses %Upper Lawagharchenakhel shows significantly difference.

Characteristics of the specific food components of female population.
It was reported that thyroid disorders in certain areas had been described to be related certain dietary vegetation grown and consumed by the population of the area. A general information on the food pattern of the female population was collected from the volunteers during the sampling time and also from the markets. The population mostly were consuming vegetables along the staple foods of wheat products.

Consumable vegetation of the study area. The type of the vegetables and their source for the availability to the populations in each locality of the Karak are presented in tab.3. Almost in every locality of the study area cabbage is the common source of consumption.

Table 4: Overall consumable vegetation in different Karak areas.

The Areas of Karak	Type of Vegetables
Zebichenakhel	Spinach, cabbage, spring onion. Mint, Broccoli, cauliflower etc
Lawagharchenakhel	Spinach , cabbage, green beens, peas, brinjal etc
Upper Lawagharchenakhel	Spring onion, mustard greens, spinach, cauliflower, leafy green etc

Consumption frequency of the vegetation: Table 4. Represents the consumption frequency of intake of edible vegetation by the female population of the Karak area. the numerical form such as 1,2, 3,+1,+2 and +3 represents the time intake habit of study subjects per week were: one time of week(1), two time of week(2), three time of week (3) and + symbol shows more than one time consumption of vegetables.

Table 5: Consumption Frequency in studies localities.

S.N0	Vegetables	Localities		
		Zebichenakhel	Lawagharchenakhel	Upper Lawagharchenakhel
1	Mustard Greens	+3	+1	+2
2	Broccoli	+2	+2	+3
3	Cauliflower	+3	+1	+3
4	Cabbage	+3	2	+3
5	Spinach	+2	2	2
6	Spring Onion	2	+2	2
7	Brinjal	2	2	2
8	Leefy Greens	+3	+3	+3
9	Pees	2	2	+2
10	Intake Frequency of Vegetable's by selected subjects / day	(+3) M.G, Caul &Cab	(+3)Leefy G	(+3)Bro, Caul, Cab &Leefy G

List of Consumption Frequency per week:
1=once a week
2= twice a week
3=three time a week
+1=more than one a week`
+2=more than twice a week
+3=more than three time a week

The consumption frequency of edible vegetation by the female population of the area has is presented in Table. 5. The vegetable consumption in Zebichenakhel varied depending on the type of vegetables. The leafy greens, such as mustard green, cauliflower, cabbage, and other greens, were consumed more than three times per week. In contrast, broccoli, spinach, spring onions, brinjal, and peas made up the majority of vegetables consumed in Lawagharchenakhel. The intake of these vegetables was highest among the selected population for this study. Next in line were spring onions and broccoli, with a consumption of +3, respectively. Other significant vegetables consumed in this location were cabbage (+2), spinach (+1), and brinjal (+0). Mustard greens and cauliflower were also consumed, but at a lower frequency. Broccoli, cauliflowers, cabbage, leafy greens (mixture), spinach, spring onions, brinjal (mixture), and peas registered as the most consumed vegetables in Upper Lawagharchenakhel locality.

Discussion:
Thyroid disorders, affecting millions worldwide (Vanderpump, 2023). Whereas, environmental factors have a crucial role in determining endocrine health, (kalra and kapoor, 2021). Thyroid disorders are a public health problem globally.
The present research has been carried in the Karak district, Khyber Pakhtunkhwa province of Pakistan. Three settlements (Zebichenakhel, Lawagharchenakhel and Upper Lawagharchenakhel) were randomly selected female population. The present study shows the highest percentage (46.1%) of euthyroid were in Lawagharchenakhel and 33.3% were present in the Upper Lawagharchenakhel. Also the euthyroid status percentage in Zebichenakhel was 18%.Additionally, the highest number of hypothyroid subjects comprising 5% were found in the Zebichenakhel. Moreover the percentage of hypothyroid status was 30.76% in Lawagharchenakhel and The lowest percentage of 13.33% present in Upper Lawagharchenakhel**.** Likewise, the percentage of hyperthyroid status was 13.33% in Upper Lawagharchenakhel, 7.69% in Lawagharchenakhel. The lowermost number of hyperthyroid subjects were observed with the percentage of 5.88% present in Zebichenakhel.
The incidence pattern indicates that hypothyroidism was more prevalent disorder. It has been observed in the studied area that the prevalence rates differ between countries, with India having a higher prevalence compared to the United Kingdom and the United States. For instance, India has a notably higher prevalence of 11%, indicating a greater number of individuals affected by thyroid issues. On the other hand, the United Kingdom and the United States of America report lower prevalence of 4.6% and 2%, respectively, suggesting that a smaller percentage of their populations are affected by thyroid dysfunction (Das *et al.*, 2022). In all these studies the incidence females was more than males (77.5%, 22.5%) and the ratio was 3.4:1. The findings revealed that hypothyroidism was more common than hyperthyroidism, with females accounting for 83.64% of hypothyroidism and males accounting for only 16.36% of hyperthyroidism (Mousa & Zoori, 2023).Women had 1.57 times the chances of euthyroidism with positive antibodies, 2.1 times the odds of subclinical hyperthyroidism, 2.37 times the odds of clinical hypothyroidism, and 1.58 times the chances of subclinical hypothyroidism as men (Strikic *et al.,* 2022).
According to Khan *et al.*, (2018), females in their study group had a 26.5% incidence rate of hypothyroidism, a common thyroid disorder characterized by an underactive thyroid gland. This figure was higher than the global incidence estimate of 5% (Khan *et al.*, 2018).
The consumable water and the food sources have great impact on the thyroid health status. Specifically iodine contents is the principal factor in water and foods that will determine the normal and dysfunctional status of the thyroid. Iodide from the ocean is changed into basic iodine, which is flammable and returns to the land and water with rain. Iodine insufficiency may result from the incomplete cycling of iodine in several locations. Foods cultivated on iodine-deficient soil are low in iodine, and people and animals that eat them get iodine deficiencies (WHO, 2007). Inland places, hilly areas, and areas that experience

regular floods are where iodine deficient soils are most prevalent, while they can also be found in coastal areas (Assey *et al.*, 2004). Iodine deficiency is the most prevalent cause of hypothyroidism globally, with higher incidence in hilly soil and near rivers due to soil leaching (Malla & Shafi, 2018).

The characteristics of the consumable water through various ingredients affect thyroid health. The pH and the conductivity also affect thyroid function discussed in the review of literature. The analysis of the consumable water in the different localities had shown that pH and the conductivity property of the water is well within the range of WHO standards. In the same pattern in the water of the study area total dissolved solids are well in the range of WHO standards. Therefore, the high frequency of thyroid problems in the Karak area cannot be attributed to pH, conductivity, or total dissolved solids characteristics.

The adequate range of iodine in the water sources as assessed by numerous samples was between 5 to 30 ug/L. In the water samples of the localities of the Karak except in the locality of Zebichenakhel in the other localities the iodine contents were in the upper side of the range. Iodine deficiency in the water may not be considered the cause of thyroid disorder in the region. While, iodine is the most crucial ingredient of thyroid hormones and is obtained from external environment to the body through consumable water and the food items. Deficiency of iodine in the body results in the reduction of T3 and T4 production. Excess and deficiency of these two hormones result in the major diseases like Hyperthyroidism and Hypothyroidism. The measurement of T3, T4 plasma level becomes essential for the identification and regular maintenance of thyroid hormones in the body and human well-being (Rajalashmi & Begam, 2021).

The fluoride in excess has been known to disrupt thyroid gland operation. Thyroid disorders brought on by fluoride that resembled iodine insufficiency (Hosur *et al.*, 2012). Two factors had been recognized for the cause of endemic goiter in the north western part of union of South Africa. Primary the lack of iodine in the soil and drinking water, as well as what are likely high levels of fluorine in underground drinking water despite the waters' abundance in iodine (Jooste *et al.*, 1999). The effects of high fluoride intake from consumable water, thyroid hormones and TSH have been studied in both humans and animals. Several studies had reported a lowering of the thyroxin and tri-iodothyronine accompanying marked increase in the TSH levels (Zeng *et al.*, 2012). The water samples of the all localities of Karak contain far less fluoride contents the permissible WHO limits. The fluoride contents of all the water samples at different localities showed the range of 0.1 to 0.2mg/L. Therefore, fluoride was ruled out as the factor for high incidence of thyroid disorders in the Karak area.

Selenium is an important micronutrient and required for thyroid functions, specifically in the metabolism of thyroid hormones in the glandular tissue specifically for the enzymes. Despite certain advantages for patients with serious diseases, selenium supplementation is not currently suggested in the prescribed course of therapy due to a lack of sufficient data (Zheng *et al.*, 2018).Selenium deficiency may have significant implications on the thyroid gland's ability to metabolize thyroid hormones (Contempre *et al.*, 2004;Thomson *et al.*, 2005). In the water samples of the localities of the Karak satisfactory quantities of selenium are detected which may not be considered as the selenium deficient status. The selenium contents of all the water samples at different localities showed the range of 0.5 to 12.2ug/L. The selenium status in the water samples of the Karak area could not be a factor for higher incidence of thyroid disorders.

Nitrates in the consumable water or foods are found to inhibit uptake of iodide by thyroid as these bind to the sodium-iodide symporter responsible of the uptake of iodide at the level of thyroid follicles (Kherad *et al.*, 2016). In agricultural regions, nitrate contaminates drinking water and is also present in high concentrations in some vegetables. Because nitrate inhibits the thyroid's ability to absorb iodide, thyroid function may be impacted. High levels of nitrates can cause hypothyroidism and thyroid cancer (Ward *et al.,* 2010). In the water samples of the localities of the Karak area nitrate contents are far less than the maximum permissible limit by WHO. The concentration of nitrate in all the water samples at different localities exhibited the range of 5 to 18mg/L. Nitrate were also ruled out for any role in high incidence of thyroid disorders in the study area.

Cyanide the other content of water may affect thyroid function although the studies on this context are lacking. The activities of FT3, FT4, and TSH were significantly different from those of the control group in an experimental research on Wistar rats (P 0.05). In contrast to the control group, which had normal thyroid architecture, the treatment group's thyroid glands had substantial epithelial hyperplasia, cellular degeneration, and sparse cytoplasm. It had been determined that cyanide caused hyperthyroidism (Tamydayo, 2014). Cyanide, nitrate and flavonoids are among the substances that endanger hormones and the human body, particularly the thyroid gland, and so have an impact on the body (Umar *et al.*, 2023). The concentration of cyanide in all the water samples at different localities exhibited the range of 8 to 55ug/L. Anyway cyanide could not be the part of the discussion as the measured content in the water samples of the Karak area contain far less than the permissible limit by WHO.

It has been reported previously that the numerous aspects of the thyroid function interference caused by diet and environment (Main *et al.*, 2022).The food items have dual influence on thyroid gland activity. On one side it may be lacking sufficient iodine necessary for adequate thyroid function, on the other side food item may contain interfering compounds which affect thyroid iodine uptake and or interfering in the organification of the iodine in thyroid tissue. These act as goitrogens (Muzzaffar *et al.*, 2022) as stated in the literature review.

The population living in the rural areas understandably rely on locally grown vegetables for their daily sustenance. In Karak population, like other areas of Pakistan, the consumption of the vegetation contain substances that affect adversely thyroid functions may be carrying goitrogens. The Karak area specific food vegetation along with the staple food of wheat is high. Almost in every locality the vegetation include cabbage, mustard green and cauliflower which commonly consumed in the area.

It is advisable to consume goitrogen-containing foods (Cruciferous vegetables, soy-based products, starchy plants) in moderation. Goitrogens, potentially leading to an increased risk of developing goiter and hypothyroidism (Djuričić *et al.*, 2022). Various investigations have reported that the exceptionally high intake of cruciferous vegetables like cabbage and turnips has been found in animals with hypothyroidism (insufficient thyroid hormone production) (Fenwick *et al.*, 1983). There are two possible explanations for this impact. Cruciferous vegetables include the compound progoitrin, which may be hydrolyzed to create goitrin, which can have an impact on thyroid hormone synthesis. One specific kind of glucosinolates, indole glucosinolates, hydrolyze to produce thiocyanate ions, which may compete with iodine for thyroid gland absorption (Felker *et al.*, 2006). However, unless iodine shortage is also present, it is not believed that increasing one's intake of cruciferous vegetables increases the risk of developing hypothyroidism. Studies on human volunteers had shown that eating cooked Brussels sprouts at a rate of 150 g per day (5 oz per day) for four weeks had no negative impact on thyroid function (McMillan *et al.,* 1986). Similar to this, eating a lot of cruciferous vegetables has only been linked to an elevated risk of thyroid cancer in locations that lack iodine (Cho and Kim, 2015).

It is thus assumed from the results that the goitrogenic effects of the contents of food vegetation consumed may be the probable cause of hypothyroidism in the area. The reason that the female gender in the area is specifically affected by this mechanism may be discussed that males of the area mostly work in the areas far from the study area and more convincingly that female reproductive physiology along the goitrogenic food consumption is the cause of hypothyroidism.
Conclusion: Interfering agents in the synthesis and secretion of thyroid gland in the food vegetation of the area has been considered the cause of the hypothyroidism. The uneven iodine supplementation in the hypothyroid inducing scenario probably the cause of the development of hyperthyroidism in the studied female population.

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