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Association Between Thyroid Function and Oxidative Stress Markers in Overt and Subclinical Hypothyroid Patients: Observational Cross-Sectional Study in Nepal

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ABSTRACT: Background: Hypothyroidism poses significant health concerns, with oxidative stress playing a potential role in its development. However, the mechanisms remain unclear. **Methods:** The study used a cross-sectional design to analyze oxidative stress markers and thyroid function parameters in adult hypothyroid patients at Chitwan Medical College, Nepal. A convenience sampling of 225 patients was used. Data collection involved questionnaires, clinical assessments, and biochemical assays to measure free tri-iodothyronine (T3), thyroxine (T4), thyroid-stimulating hormone (TSH), and superoxide dismutase (SOD) enzyme from serum and malondialdehyde (MDA), glutathione peroxidase (GPx), and total antioxidant capacity (TAC) from plasma. Descriptive statistics, Pearson correlational, and linear regression analyses were performed using IBM SPSS (version 20). **Results:** Subclinical hypothyroidism was most common among overweight individuals aged 46-60, especially in females (117 out of 167). Significant differences ($p < 0.001$) were observed between subclinical and overt hypothyroid patients in thyroid profiles and oxidative stress parameters. Thyroid-stimulating hormone (TSH) levels showed a positive correlation with glutathione peroxidase (GPx) and malondialdehyde (MDA) and a negative correlation with Superoxide dismutase (SOD) and total antioxidant capacity TAC in both overt and subclinical patients. **Conclusion:** Hence the subclinical hypothyroidism, prevalent in overweight individuals aged 46-60, particularly in females, is very high compared to overt hypothyroidism, where TSH was positively correlated with the Glutathione peroxidase (GPx) and Malondialdehyde (MDA), representing increased TSH levels are associated with increased oxidative stress in the body.

Keywords: Hypothyroidism, Oxidative stress, Thyroid hormones, Overt Hypothyroidism, Sub-clinical Hypothyroidism

INTRODUCTION

Subclinical and overt forms of hypothyroidism are a common endocrine disorder. In subclinical hypothyroidism, T4 and T3 levels of thyroid hormones are normal, but the thyroid-stimulating hormone (TSH) is up-regulated. (1) In Overt hypothyroidism, TSH is high while T4 is low. The blood's T3, T4, and TSH levels indicate thyroid function. Metabolism and energy production are regulated by two hormones known as tri-iodothyronine (T3) and thyroxin (T4). The hypothalamus produces thyrotropin-releasing hormone (TRH), which further stimulates the pituitary gland to release thyroid-stimulating hormone (TSH), and the TSH signals the thyroid gland to produce and release T3 and T4. (2) The thyroid gland dysfunction outcomes various other diseases, such as heart diseases, metabolic diseases, depression, etc., strongly indicating the need for proper thyroid activity regulation. (3) The rate of thyroid disorders has recently gone up, and this has raised many concerns for public health. More so, women, especially those of child-bearing age, are affected most by the maternity leave issue, for instance. Some of the causes of hypothyroidism include Autoimmunity, Iodine deficiency, Genetic predisposition, Environment through pollutant agents, and medications (4). A meta-analysis study documented overt hypothyroidism of 0. 37% and subclinical cases of 3.8% in Europe, with a total Hypothyroidism count of 12.41% in residents of the western region of Nepal. (5) (6) Thyroid dysfunction is capable of causing oxidative stress where the body's antioxidants do not balance the creation of ROS (7) Lipid peroxidation products are biomarkers of oxidative damage and may play a role in the development of hypothyroidism (7). Some recent studies have indicated positive cross-sectional associations between thyroid functioning and oxidative stress markers. (8) As thyroid hormones are implicated in controlling the products of cellular metabolism and antioxidant protection, the condition of oxidative stress may negatively affect the production of THS and related signaling. Its understanding is critical in explaining the mechanisms that implicate thyroid dysfunction and oxidative stress in causing the condition(9). While TSH levels and other thyroid hormones have been generally found to be modulators and reflect oxidative stress activity, there are few data regarding the mechanisms and therapeutic aspects.(10) Hence, the scope of the present research is to offer a broad analysis of the relationship between thyroid function variables (T3, T4, TSH) and oxidant damage markers. By adopting a cross-sectional quantitative study design, the study intends to identify possible biomarkers of oxidant stress in subclinical, overt and inconspicuous hypothyroid subjects and to discuss their relevance with respect to hypothyroidism treatment strategies.

MATERIALS AND METHOD

Study design and population

The present study employed hypothyroid participants visiting in endocrinology Outpatient Department (OPD) at Chitwan Medical

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College and Teaching Hospital in Bharatpur, Nepal, for their routine annual clinical examination following eligibility screening based on predefined inclusion and exclusion criteria. Eligible patients were approached and provided with detail information of the study, including its objectives, procedure, risks and benefits. Written consent was received from each patient willing to participate. The confidence level (Z) of the study was 95 % and the allowable error margin (d) was 5%. This method employ Cohran (1963) formula for calculation of sample size.(6), thus taking into consideration the 12.41% prevalence rate (P) of hypothyroid cases in previous report the sample size was more than 165 was established.

$$\text{Sample size (n)} = Z2\ P\ (1\text{-}P) / d2$$

Inclusion and Exclusion criteria

The inclusion criteria included written informed consent, being a confirmed hypothyroid patient, and being an adult and greater than or equal to 15 years of age. The exclusion criteria included patients with hyperthyroidism, any abnormal dietary habits, patients on any thyroxin, anti-thyroid and lipid lowering medication or antioxidant vitamin supplements that may interfere with thyroid function and oxidative status markers.

Physical body evaluation and biochemical analysis

Hypothyroid participants name, age and sex were obtained through questionares, their weight and height were measured to evaluate the body mass index (BMI). Afterward, participants blood samples were collected, and serum and plasma were subsequently isolated and analyzed for their thyroid profile, superoxide dismutase enzyme (SOD) and malondialdehyde (MDA) levels, glutathione peroxidase (GPx), and total antioxidant capacity (TAC). Free T3, T4, and TSH levels were quantitatively determined using direct Chemiluminescent technology on the Siemens ADVIA Centaur XP (11). The levels of Malondialdehyde (MDA), total antioxidant capacity (TAC), glutathione peroxidase (GPx) enzyme, and superoxide dismutase (SOD) enzyme were quantified following the methods outlined by Jean CD et al.(1983), Benzie.F.F and J.J strain (1996), Hafeman DG et al.(1974) and Mishra HP, Fridovich I (1972)(12) (13) (14) (15) (16).

Statistical analysis

Statistical analysis was conducted using IBM SPSS (version 20). This analysis included descriptive and analytical statistics. Pearson's correlation was applied to determine if there is an association between thyroid function parameters (T3,T4,TSH) and oxidative stress markers (MDA, TAC, GPx, SOD). A two tailed test with significance levels of 0.05 and 0.01 was used. Simple linear regressions were performed to assess the extent to which specific thyroid function parameters in different types of hypothyroid patients can explain variance in oxidative stress.

Ethical consideration

Before collecting data, ethical approval was granted by the Chitwan Medical College Institutional Review Committee with code number: 2078/79-231. The study followed the ethical guidelines specified in the Helsinki Declaration. Confidentiality and anonymity were ensured through the acquisition of informed consent. Referrals to treating physicians were made in response to abnormal results.

Limitations of the study

The limitation of this present study includes recruitment of the patients from a single institution, calculation of sample size was based on 12.41% prevalence rate and exclusion of patients receiving medication including thyroxin, anti-thyroxin, lipid lowering drugs and antioxidant vitamin supplements.

RESULTS

As indicated in or overt hypothyroidism (n=11). Additionally, Table 1 shows distribution of BMI categories among hypothyroidism subjects. Among those with sub-clinical hypothyroidism, the highest frequency (n= 54) fell within the overweight category while the lowest frequency (n=1) was observed in the second-grade obesity category. Among those with the over hypothyroidism, the highest frequency (n=22) fell within the normal weight category, while the lowest frequency (n=11) was observed in the underweight obesity category.

Table (1, the study included 152 sub-clinical hypothyroidism patients (117 females, 35 males) and 73 overt hypothyroidism patients (49 females, 24 males). The age group with the highest frequency was 46-60 years for sub-clinical hypothyroidism (n= 54) and 31-45 years for overt hypothyroidism (n=22). The lowest frequency was observed in the age group of 76 and above for sub-clinical hypothyroidism (n=1) and 15-30 years for overt hypothyroidism (n=11). Additionally, Table 1 shows distribution of BMI categories among hypothyroidism subjects. Among those with sub-clinical hypothyroidism, the highest frequency (n= 54) fell within the overweight category while the lowest frequency (n=1) was observed in the second-grade obesity category. Among those with the over hypothyroidism, the highest frequency (n=22) fell within the normal weight category, while the lowest frequency (n=11) was observed in the underweight obesity category.

Table (1). Demographic characteristics of the study groups.

Parameters	Thyroid disorder groups Frequency		P Value
	Sub-Clinical Hypothyroidism Patients Quantity	Overt Hypothyroidism patients Quantity	
Sex			0.116
Female	117	49	
Male	35	24	
Total	152	73	
Age (years)			
15-30	20	11	0.000
31-45	48	22	
46-60	54	14	
61-75	29	12	
76 and above	1	14	
Body Mass Index (Wt (kg)/Ht (m2)			
Under-Weight (<18.5)	20	11	0.000
Normal Weight (18.5-24.9)	48	22	
Overweight (25-29.9)	54	14	
First Grade Obesity (30–34.9)	29	12	
Second Grade Obesity (35-39.9)	1	14	

As shown in Table 2, clinical variables were compared between patients with subclinical hypothyroidism and overt hypothyroidism. It was observed that mean levels of thyroid hormones, T3 (2.88±0.38 vs. 1.66±0.58, $p<0.001$) and T4 (1.03±0.13 vs. 0.56±0.28, $p<0.001$), were significantly lower in patients with overt hypothyroidism, while TSH levels (0.15±5.97 vs. 63.89±51.94, $p<0.001$) were significantly higher in overt hypothyroidism. Oxidative stress markers showed a different pattern. MDA levels (2.38±0.31 vs. 3.63±1.31, $p<0.001$) were found to be higher in overt hypothyroidism, suggesting that oxidative stress is more pronounced in overt hypothyroidism. However, antioxidant enzyme activities, including SOD (8.61±0.31 vs. 7.28±2.13, $p<0.001$), and TAC (638.56±13.35 vs. 536.71±52.60, $p<0.001$), were

significantly lower in overt hypothyroidism, suggesting that the antioxidant defense system is compromised in overt hypothyroidism. On the other hand, GPx enzyme (13.41±1.47 vs. 21.48±7.86, p<0.001) was found to be elevated in overt hypothyroidism.

Table (2): Descriptive statistics for clinical variables among hypothyroid patients.

Parameters	Sub-Clinical Hypothyroidism	Overt Hypothyroidism	P value
	Mean±SD	Mean (SD)	< 0.001
T3 (pgm/ml)	2.88±0.38	1.66±0.58	< 0.001
T4 (ngm/ ml)	1.03±0.13	0.56±0.28	< 0.001
TSH (µIU/ml)	0.15±5.97	63.89±51.94	< 0.001
MDA (µmol/L)	2.38±0.31	3.63±1.31	< 0.001
SOD (U/gm P/mL)	8.61 ±0,31	7.28±2.13	< 0.001
GPx (U/mg of Hb)	13.41±1.47	21.48±7.86	< 0.001
TAC (µmol/L)	638.56±13.35	536.71±52.60	< 0.001

The correlation matrix, depicted in Table (3, revealed significant associations between oxidative stress markers and thyroid function parameters among hypothyroid patients. A strong positive correlation was observed between MDA levels and TSH (r=0.987, p<0.01), indicating increased oxidative damage with worsening hypothyroidism, while substantial negative correlation between MDA and T3 (r = -0.679, p < 0.01), MDA and T4 (r = -0.678, p < 0.01) were observed. Furthermore, significant positive correlations were identified between SOD and T3 (r = 0.580, p < 0.01), SOD and T4 (r = 0.594, p < 0.01), and a negative correlation between SOD and TSH (r = -0.967, p < 0.01). Similarly, GPx exhibited substantial negative correlations with T3 (r = -0.698, p < 0.01), T4 (r = -0.689, p < 0.01), and a highly positive correlation with TSH (r = 0.986, p < 0.01). Additionally, TAC demonstrated significant positive correlations with T3 (r = 0.800, p < 0.01) and T4 (r = 0.784, p < 0.01), alongside a notable negative correlation with TSH (r = -0.953, p < 0.01).

Table (3): Correlation matrix for oxidative stress markers and thyroid function parameters among hypothyroid Patients

	T3	T4	TSH
MDA	-0.679**	-0.678**	0.987**
SOD	0.580**	0.594**	-0.967**
GPx	-0.698**	-0.689**	0.986**
TAC	0.800**	0.784**	-0.953**

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

In

Table (**4** shows the detailed relationship (r values) between various thyroid hormones and oxidative stress markers in subclinical hypothyroid patients, while Figure (1, Figure (2, and Figure (**3** depict the corresponding regression coefficient R square values. Malondialdehyde (MDA) exhibited a negative correlation with both T3 (r = -0.164, p < 0.01) and T4 (r = -0.154, p < 0.05), with R2 values of 0.027 and 0.024, respectively, as shown in Figure (1 and Figure (2. Conversely, MDA displayed a notably positive correlation with TSH (r = 0.938, p < 0.01), as depicted by an R2 value of 0.881 in Figure (**3**. Similarly, Superoxide dismutase (SOD) demonstrated a positive correlation with T3 (r = 0.161, p < 0.05) with an R2 value of 0.026 in Figure (1 and T4 (r = 0.151, p > 0.05) with an R2 value of 0.023 in Figure (2, with a strong negative correlation observed with TSH (r = -0.938, p < 0.01) and an R2 value of 0.881 in Figure (**3**. Glutathione Peroxidase (GPx) exhibited negative correlations with T3 (r = -0.192, p < 0.05) with an R2 value of 0.037 in Figure (1 and T4 (r = -0.109, p > 0.05) with an R2 value of 0.012 in Figure (2 while showing a positive correlation with TSH (r = 0.834, p < 0.01) and an R2 value of 0.696 in Figure (**3**. Moreover, Total Antioxidant Capacity (TAC) displayed positive correlations with both T3 (r = 0.204, p < 0.05) with an R2 value of 0.042 in Figure (1 and T4 (r = 0.164, p < 0.05) with an R2 value of 0.027 in Figure (2, alongside a strong negative correlation with TSH (r = -0.998, p < 0.01) and an R2 value of 0.997 in Figure (**3**.

Table (4): Correlation matrix for oxidative stress markers and thyroid function parameters among Subclinical hypothyroid patients.

	T3	T4	TSH
MDA	-.164**	-.154*	.938**
SOD	.161*	.151	-.938**
GPx	-.192*	-.109	.834**
TAC	.204*	.164*	-.998**

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

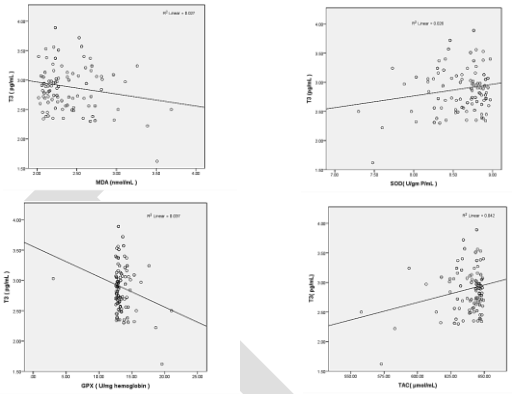


Figure (1): T3 vs. MDA, T3 vs. SOD, T3 vs. GPx, T3 vs. TAC (Subclinical hypothyroid patients).

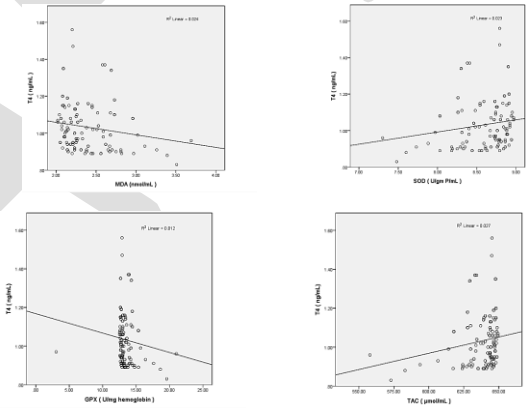


Figure (2): T4 vs MDA, T4 vs SOD, T4 vs GPx, T4 vs TAC (Subclinical hypothyroid patients).

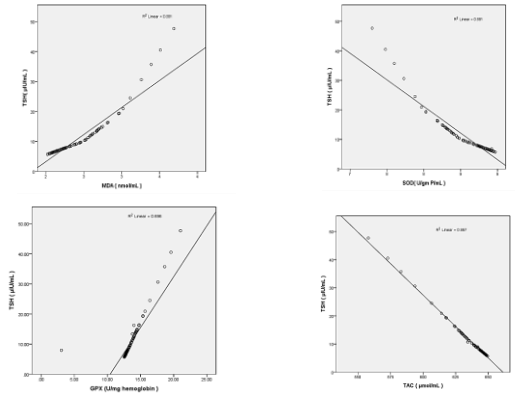


Figure (3). TSH vs. MDA, TSH vs. SOD, TSH vs GPx, TSH vs TAC (Subclinical hypothyroid patients).

Table 5 presents the detailed relationship (r values) between various thyroid hormones and oxidative stress markers in overt hypothyroid patients, while Figures 4 and 6 depict the corresponding regression coefficient R square values. Table (**5** illustrates that Malondialdehyde (MDA) exhibited a strong negative correlation with

T3 (r = -0.566, p < 0.01) with an R2 of 0.321 in Figure (**4** and T4 (r = -0.524, p < 0.01) with an R2 of 0.275 in figure 5, while displaying a highly positive correlation with TSH (r = 0.998, p < 0.01) with an R2 of 0.997) in Figure 6. Conversely, Superoxide dismutase (SOD) demonstrated strong positive correlations with both T3 (r = 0.548, p < 0.01, R2 0.301 in Figure 4 and T4 (r = 0.519, p < 0.01 R2 of 0.270 in Figure 5, along with a pronounced negative correlation with TSH (r = -0.984, p < 0.01, R2 of 0.989 in figure 6. Glutathione peroxidase (GPx) displayed significant negative correlations with T3 (r = -0.552, p < 0.01, R2 0.305 in Figure 4 and T4 (r = -0.509, p < 0.01, R2 0.260) in Figure 5, while exhibiting a highly positive correlation with TSH (r = 0.987, p < 0.01, R2 of 0.975 in Figure 6. Additionally, total Antioxidant Capacity (TAC) showed positive correlations with both T3 (r = 0.562, p < 0.01, R2 0.319 in Figure 4 and T4 (r = 0.529, p < 0.01 R2 0.280 in Figure 5, alongside a perfect negative correlation with TSH (r = -1.00, p < 0.01, R2 1 in Figure 6.

Table (5): Correlation matrix for oxidative stress markers and thyroid function parameters among overt hypothyroid patients

	T3	T4	TSH
MDA	-.566**	-.524**	.998**
SOD	.548**	.519**	-.984**
GPx	-.552**	-.509**	.987**
TAC	.562**	.529**	-1.00**

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

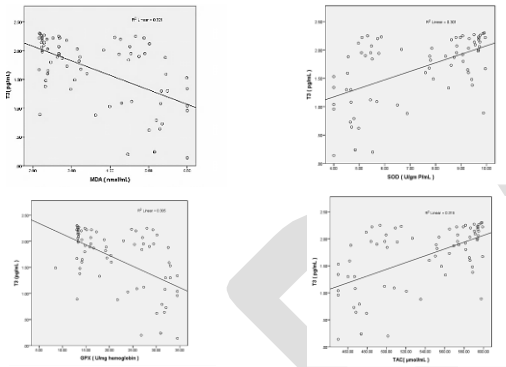


Figure (4): T3 vs MDA, T3 vs SOD, T3 vs. GPx, T3 vs. TAC (Overt hypothyroid patients).

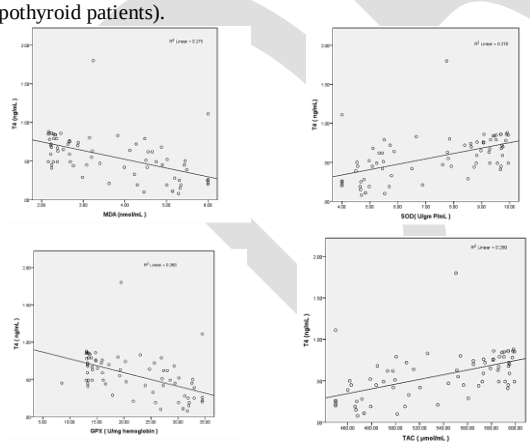
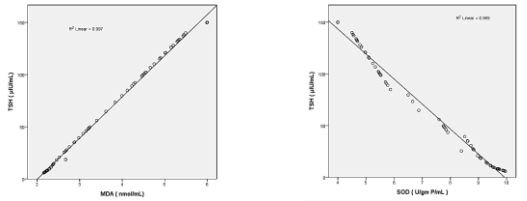


Figure (5): T4 vs MDA, T4 vs SOD, T4 vs. GPx, T4 vs. TAC (Overt hypothyroid patients).



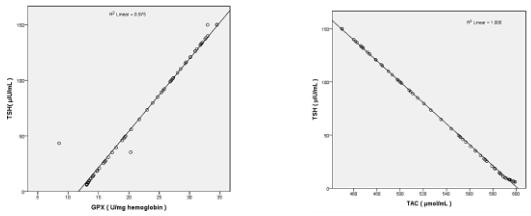


Figure (6): TSH vs MDA, TSH vs. SOD, TSH vs. GPx, TSH vs TAC (Overt hypothyroid patients).

DISCUSSION

The present study investigated the demographic characteristics of patients diagnosed with sub-clinical and overt hypothyroidism, which was conducted at the endocrinology outpatient department of Chitwan Medical College and Teaching Hospital in Bharatpur, Nepal. Here, hypothyroid patients attending the endocrinology OPD for routine clinical examination were enrolled following eligibility screening on predefined inclusion and exclusion criteria. At the same time, this recruitment method provided a targeted sample of hypothyroid patients, so it may have introduced a potential limitation by over-representing individuals with more severe or chronic forms of hypothyroidism. This could potentially introduce bias, limiting the generalizability of the finding to the broader hypothyroid population, specifically those with milder or less chronic conditions, as in our present study, or overt hypothyroidism (n=11). Additionally, Table 1 shows distribution of BMI categories among hypothyroidism subjects. Among those with sub-clinical hypothyroidism, the highest frequency (n= 54) fell within the overweight category while the lowest frequency (n=1) was observed in the second-grade obesity category. Among those with the over hypothyroidism, the highest frequency (n=22) fell within the normal weight category, while the lowest frequency (n=11) was observed in the underweight obesity category.

Table (1 displayed a higher prevalence of subclinical hypothyroidism compared to overt hypothyroidism, with 152 and 73 patients, respectively, which aligns with previous research suggesting that subclinical hypothyroidism is more prevalent in the general population than overt hypothyroidism. (17) or overt hypothyroidism (n=11). Additionally, Table 1 shows distribution of BMI categories among hypothyroidism subjects. Among those with sub-clinical hypothyroidism, the highest frequency (n= 54) fell within the overweight category while the lowest frequency (n=1) was observed in the second-grade obesity category. Among those with the over hypothyroidism, the highest frequency (n=22) fell within the normal weight category, while the lowest frequency (n=11) was observed in the underweight obesity category.

Table (1 shows the age distribution differed between the two groups, with the age group of 46-60 years showing the highest frequency among sub-clinical hypothyroidism patients, while the age group of 31-45 years exhibited the highest frequency among overt hypothyroidism patients, which supports with previous studies highlighting age as a significant risk factor for hypothyroidism (18). The prevalence of subclinical hypothyroidism tends to increase with age, possibly due to age-related changes in thyroid function and an increased susceptibility to thyroid disorders. (17) Furthermore, the present study indicated disparities in gender within the distribution of hypothyroidism patients, with a higher proportion of females than males in both sub-clinical and overt hypothyroidism groups. This gender difference is consistent with existing literature, which suggests that females are more susceptible to thyroid disorders compared to males (19). The hormonal fluctuations associated with female reproductive health, such as pregnancy and menopause, may contribute to the higher prevalence of thyroid dysfunction among women (20).

Table (1) revealed intriguing insights into the association between thyroid dysfunction and weight status among the studied population. The results indicate that overweight (25-29.9) was the most prevalent

BMI category among subclinical hypothyroidism patients, with 54 individuals falling into this category, whereas normal weight (18.5 – 24.9) was the most common category among overt hypothyroidism patients, comprising 22 individuals. This contrasts with previous studies suggesting a higher prevalence of obesity among individuals with overt hypothyroidism (21). Furthermore, the findings in Table (2 findings demonstrated significant differences in thyroid hormone levels between the two groups. Sub-clinical hypothyroidism patients exhibited higher mean levels of T3 and T4 compared to those with overt hypothyroidism. Conversely, TSH levels were markedly elevated in the overt hypothyroidism group. These results align with established diagnostic criteria for subclinical and overt hypothyroidism, highlighting the importance of thyroid hormone and TSH measurements in the classification and management of thyroid disorders. (22) This shows that overt hypothyroidism patients exhibited significantly higher levels of Malondialdehyde (MDA) compared to subclinical hypothyroidism patients. This suggests a greater burden of oxidative stress in overt hypothyroidism, potentially contributing to the pathogenesis of hypothyroidism-related complications. Conversely, antioxidant enzyme activities, including Superoxide Dismutase (SOD) and Glutathione Peroxidase (GPx), were found to be significantly lower in overt hypothyroidism patients compared to those with subclinical hypothyroidism. This indicates a compromised antioxidant defense system in overt hypothyroidism, potentially exacerbating oxidative stress-induced cellular damage. Moreover, Total Antioxidant Capacity (TAC) was also significantly reduced in overt hypothyroidism patients, further highlighting the dysregulation of antioxidant defense mechanisms in this population. Table (3 findings represented the associations between oxidative stress markers and thyroid function parameters among hypothyroid patients, revealing a positive correlation between malondialdehyde (MDA) levels and thyroid-stimulating hormone (TSH) levels. This observation suggests that increased oxidative damage, as evidenced by elevated MDA levels, corresponds with the severity of hypothyroidism. This finding aligns with previous studies demonstrating the role of oxidative stress in thyroid dysfunction (23). The significant negative correlations between MDA and triiodothyronine (T3), as well as MDA and thyroxine (T4), emphasize the inverse relationship between oxidative stress and thyroid hormone levels, highlighting the potential impact of oxidative stress on thyroid hormone synthesis and metabolism. Furthermore, the present study identified significant positive correlations between superoxide dismutase (SOD) levels and both T3 and T4 levels. This suggests that higher SOD activity is associated with increased levels of active thyroid hormones, indicating a potential protective effect against thyroid dysfunction. However, the negative correlation between SOD and TSH levels implies that SOD activity may decrease with worsening hypothyroidism, potentially contributing to the buildup of oxidative damage. Table 3 shows that glutathione peroxidase (GPx) levels exhibited substantial negative correlations with T3 and T4 levels while showing a strong positive correlation with TSH levels. Additionally, total antioxidant capacity (TAC) has significant positive correlations with T3 and T4 levels and a notable negative correlation with TSH levels. This implies that higher antioxidant capacity is associated with increased thyroid hormone levels and reduced TSH levels, highlighting the potential protective role of antioxidants against hypothyroidism-induced oxidative stress.

We are further evaluating the relationship between oxidative stress markers and thyroid function parameters among subclinical hypothyroid patients. Table (**4**, Figure (1, and Figure (2 depict a negative correlation between malondialdehyde (MDA) levels and both T3 and T4 levels, implying a potential role of oxidative stress in impairing thyroid hormone metabolism among subclinical hypothyroid patients. The present finding is consistent with previous literature suggesting that oxidative stress may contribute to thyroid dysfunction by disrupting thyroid hormone production or peripheral conversion. (23) Table (**4** and Figure (**3** demonstrate a positive correlation between MDA levels and TSH levels, suggesting a potential link between oxidative stress and TSH secretion in subclinical hypothyroidism. Elevated TSH levels, characteristic of subclinical hypothyroidism, may stimulate thyroid follicular cells to produce more hydrogen peroxide, increasing oxidative stress (24).

As reported, euthyroid patients were reported to show stable levels of SOD, GPx, and TAC, with less pronounced associations between these antioxidant and thyroid hormones. Research carried out in previous studies found that euthyroid patients have high amounts of TAC levels compared with the present studies (25). Both SOD and GPx activities are generally higher in euthyroid patients compared to overt hypothyroidism patients and closer to the levels seen in subclinical hypothyroidism (25). However, Figure (1 and Figure (2 show a positive correlation between superoxide dismutase (SOD) levels and both T3 and T4 levels, suggesting a potential protective effect of antioxidant enzymes against thyroid dysfunction in subclinical hypothyroidism. SOD, as a key antioxidant enzyme, plays a crucial role in scavenging superoxide radicals and maintaining cellular redox balance. (26) Moreover, in Figure (1 and Figure (2, the negative correlation between GPx levels and both T3 and T4 levels suggests a potential role of GPx in modulating thyroid hormone metabolism. Conversely, the positive correlation between GPx levels and TSH levels underscores the influence of thyroid status on antioxidant enzyme activity. The positive correlations between TAC levels and both T3 and T4 levels, alongside the negative correlation with TSH levels, highlight the overall antioxidant capacity concerning thyroid function among subclinical hypothyroid patients. Furthermore, Table (**5** unveils associations between oxidative stress markers and thyroid hormones in the context of overt hypothyroidism. Figure (**4** demonstrates strong negative correlations between malondialdehyde (MDA) levels and T3 and T4 levels, underscoring the pronounced impact of oxidative stress on thyroid hormone synthesis and metabolism in overt hypothyroid patients. This aligns with previous studies indicating increased oxidative stress and lipid peroxidation in overt hypothyroidism (9). Figure (**6** shows a highly positive correlation between MDA and TSH levels, suggesting a reciprocal relationship between oxidative stress and TSH secretion in overt hypothyroidism. Elevated TSH levels, characteristic of overt hypothyroidism, may exacerbate oxidative stress by promoting thyroid follicular cell hypertrophy and increasing reactive oxygen species production (9). Similarly, the strong positive correlations between superoxide dismutase (SOD) levels and both T3 and T4 levels highlight the potential compensatory response of antioxidant enzymes to mitigate oxidative damage in overt hypothyroidism. Moreover, the negative correlations between GPx levels and both T3 and T4 levels suggest a potential role of GPx in modulating thyroid hormone metabolism and protecting against oxidative stress-induced thyroid damage. Conversely, the highly positive correlation between GPx levels and TSH levels underscores the influence of thyroid status on antioxidant enzyme activity in overt hypothyroidism. The strong positive correlations between TAC levels and both T3 and T4 levels, alongside the perfect negative correlation with TSH levels, highlight the overall antioxidant capacity with thyroid function among overt hypothyroid patients.

Hence, the relationship between oxidative stress and hypothyroidism reveals a complex interplay where oxidative stress both contributes to and results from thyroid dysfunction. In hypothyroidism, reduced thyroid hormone levels impair cellular metabolism, increasing reactive oxygen species (ROS) and exacerbating oxidative damage (27). This is particularly evident in overt hypothyroidism, where oxidative stress further impairs thyroid function. Conversely, oxidative stress itself can damage thyroid cells, triggering or accelerating hypothyroidism, particularly in subclinical cases. While antioxidant defenses may offer protection in the early stages, they become overwhelmed as the condition progresses, leading to a vicious cycle of worsening oxidative damage and thyroid dysfunction (7).

CONCLUSIONS

The present study delved into the demographic profiles of patients diagnosed with sub-clinical and overt hypothyroidism, shedding light on significant associations between thyroid dysfunction, age, gender, and weight status. Furthermore, our findings underscored the intricate interplay between oxidative stress markers and thyroid function parameters, revealing potential mechanisms underlying hypothyroidism pathogenesis. Notably, sub-clinical hypothyroidism exhibited a higher prevalence than overt hypothyroidism, with distinct age and gender distributions, particularly prevalent in overweight individuals aged 46-60, exhibited notable correlations between TSH levels and oxidative stress markers, highlighting potential implications for thyroid function and oxidative status in this population. Specifically, elevated malondialdehyde levels were associated with impaired thyroid hormone metabolism, while antioxidant enzyme activities exhibited contrasting correlations with thyroid hormone levels and TSH secretion. These insights offer valuable implications for understanding hypothyroidism's multifaceted nature and underscore the importance of considering oxidative stress in its management and therapeutic interventions.

Abbreviations

T3: Tri-iodothyronine

T4: Tetra-iodothyronine

TSH: Thyroid-stimulating hormone

BMI: Body mass index

ROS: Reactive oxygen species

MDA: Malondialdehyde

TAC: Total antioxidant capacity

GPx: Glutathione peroxidase

SOD: Superoxide dismutase

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Conflicts of interest

The authors declare no conflict of interest concerning the information reported in this paper.

Ethical approval and consent to participate

Before collecting data, ethical approval was granted by the Chitwan Medical College Institutional Review Committee (reference number: 2078/79-231). The study followed the ethical guidelines specified in the Helsinki Declaration. Confidentiality and anonymity were ensured by acquiring informed consent from individual patients. Referrals to treating physicians were made in response to abnormal results.

Availability of data and materials

Raw data will be available when requested by the respected author.

Author's contribution

Fuleshwar Mandal (Concept and Design; Statistical Analysis; Writing; Revision and Editing; Final Approval), Mohd Babu Khan (Concept and Design, Final Approval), Sindhu KC (Experiment; Statistical Analysis; Writing; Revision and Editing; Final Approval), Sanjay Ray Yadav (Experiment; Revision and Editing; Final Approval), Sanjay Kumar (Experiment; Revision and Editing; Final Approval), Pankaj Kumar Mishra (Experiment; Revision and Editing; Final Approval), Shila Shrestha (Experiment; Revision and Editing; Final Approval), Ram Kishor Yadav (Experiment; Revision and Editing; Final Approval).

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