



## Impact of iodine supplementation and mtDNA mutations on papillary thyroid cancer in saudi women following a vegetarian diet

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### ABSTRACT

This study examined the influence of iodine supplementation and mitochondrial DNA (mtDNA) mutations in Saudi vegetarian women with papillary thyroid cancer (PTC). Blood and tissue samples from PTC-diagnosed women were analyzed for thyroid function, mtDNA mutations, and immunohistological features. Statistical analysis using Sigmasat was employed to compare thyroid hormone levels and mtDNA mutations between groups. Serum total levels of tri-iodothyronine and thyroid-stimulating hormone were significantly different in patients following a vegetarian diet ( $P < 0.05$ ). Patients with PTC showed an increased frequency of mtDNA mutations in the D-loop region, with significantly higher mutation rates observed in patients following a vegetarian diet compared to other PTC patient groups ( $P < 0.001$ ) and controls ( $P < 0.01$ ). Notably, the mutations were predominantly somatic in Group 3 and germline in Groups 1 and 2. The findings suggest a possible link between iodine deficiency and accelerated PTC tumorigenesis. Furthermore, mtDNA mutations may serve as potential biomarkers for the diagnosis and prognosis of PTC.

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### Introduction

Thyroid cancer is ranked the third most common cancer in Saudi Arabia, contributing to an incidence of 0.75% and a mortality of 2.0% among all cancers in 2020 (1). The disease has a female predominance, with an incidence rate of 12.9 per 100,000 among women, compared to 4 per 100,000 among men (1). Among all forms of thyroid cancer, papillary thyroid carcinoma (PTC) is the most common malignancy (2). Recent literature and analyses indicate an increase in the incidence rates of thyroid cancer in Saudi Arabia (3-5). A systematic review and meta-analysis by Alqahtani et al. highlights the recent increasing trends of PTC in Saudi Arabia and states that the important risk factors are older age, iodine deficiency, radiation exposure, and high leptin levels (5). Iodine is one of the essential trace elements involved in thyroid hormone biosynthesis (6). The thyroid hormone, thyroxine (tetra-iodothyronine, or T4) is converted to tri-iodothyronine (T3), an active metabolite. Iodine gets oxidized and trapped in the thyroid gland and binds to the tyrosine molecule of the

thyroglobulin to synthesize T3 and T4 (6). Further, iodine reduction affects thyroid function by reducing thyroid hormone secretions (T3 and T4) that increase the response of thyrotropin, the thyroid-stimulating hormone (TSH) (7). During developmental stages, such as the utero and lactation stages, T3 and T4 are highly important for adequate neural establishment and normal metabolism (6). Iodine deficiency leads to increased release of TSH, potentially resulting in such conditions as hypertrophy and hyperplasia of follicular cells. Prolonged hyperplasia may eventually progress to neoplasia leading to a risk of TC, especially among females (8).

Circulating cell-free DNA (cfDNA) is released by tumors or the surrounding tissues subjected to factors associated with cancer development, such as malnutrition and hypoxia. The cfDNA are of two forms: nuclear DNA (cf-nDNA) and mitochondrial DNA (cf-mtDNA), derived from the nucleus or cytoplasmic mitochondria, respectively. The cfDNA is gaining importance in cancer investigation. It has been found that mtDNA has several advantages over nuclear (nDNA). They are relatively

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shorter than nDNA with a simple molecular structure and carry their extrachromosomal genome with a high copy number, having the ability to replicate independently of nDNA (9). Further, mtDNA serves as a liquid biopsy and somatic mtDNA alterations are considered key targets for cancer diagnosis and prognosis. Most mtDNAs have a displacement loop (D-loop) region in the central non-coding region. D-loop is a triple-stranded region formed by the stable incorporation of a third, short 7S DNA strand (10, 11). Although the role of the D-Loop region has not been entirely elucidated, studies show that mutations in proximity to the replication and transcriptional regulators in the mitochondrial genome located in the D-Loop region may reduce the mtDNA copy number (12). The results of a study investigating the levels of cfDNA in PTC patients demonstrated a significant decrease in the plasma concentration of mtDNA (13). Reports suggest that mtDNA mutations are associated with thyroid cancer (14, 15) and could be potential genetic signatures of mitochondrial dysfunction in tumor tissues (14). Likewise, iodine deficiency is associated with carcinogenesis and mutations at the mtDNA level are considered a significant risk factor in the prevalent cases of thyroid dysfunctions and complications (16). With this background, we aim to examine the association between iodine deficiency and mtDNA mutations among women diagnosed with PTC in Saudi Arabia.

## Materials and Methods

### Study Population and Study Setting

All cases in this study were Saudi Arabian women diagnosed with PTC. The controls were healthy Saudi Arabian women. The cases and the controls were recruited in collaboration with King Fahad Medical City (KFMC), and the study was conducted between August 2020 and November 2020. Three hundred ninety-eight female patients above the age of 35 years were diagnosed with PTC, and 100 healthy controls were recruited using the convenience sampling method. Healthy participants were grouped as Controls (Group 1: C, they followed a normal balanced diet, and included both omnivores and vegetarians). The patients were grouped based on their dietary habits as follows: i) 137 patients following a non-vegetarian diet (Group 2: PTC + NVD); ii) 119 patients with standard vegetarian eating habits (Group 3: PTC + VD); and iii) 142 patients following a vegetarian diet and taking iodine supplements (Group 4: PTC + VD + I). Patients in Group 3 followed a standard vegetarian diet with a daily intake of vegetables, fruits, dairy alternatives, vegetable sources of protein, starchy carbohydrates, beans, pulses, low-fat foods, and low-sugar and low-salt staples for a period of time (6-8 months), without iodine supplement intake. Patients in Group 4 followed a vegetarian diet as well as an iodine supplement prescribed by a nutritionist. The dose of iodine supplement the patients were taking varied according to each patient's individual need. No medical therapy was provided to the participants before thyroidectomy.

ding to each patient's individual need. No medical therapy was provided to the participants before thyroidectomy.

### Inclusion and exclusion criteria

All eligible participants who had consented to the histological examinations and procedures were included in the study. None of the patients with thyroid cancer had chemotherapy or radiotherapy before their recruitment. Patients with malignancies other than thyroid cancer or those exposed to the standard neoadjuvant/adjuvant chemotherapy/radiotherapy or who were taking anti-inflammatory and immunosuppressive agents during the last three months before this research were excluded. Participants who were pregnant, or had infections or inflammatory illness during the last three months before the study, or those with a history of alcohol drinking, smoking, or drug abuse were also excluded (to avoid any other factors that could affect the findings of our study because these factors have a direct cellular toxicity on thyroid cells and may affect thyroid function).

### Thyroid Profiles

Blood samples were collected prior to the thyroidectomy. A volume of 5 ml blood sample was collected following overnight fasting to determine the thyroid hormone levels. The samples were incubated at room temperature for 10 min and were then centrifuged at 3,000 rpm for 8 min. The serum was stored at -80°C until further use. The DiaSorin kit was used to determine the serum levels of total T4, total T3, and TSH. The laboratory reference ranges were 0.5-4 mU/l for TSH, 1.2-2.8 nmol/l for T3, and 80-150 nmol/l for T4.

### DNA Extraction and Sequencing:

For DNA extraction, 3 ml of blood was collected from all 498 participants before any therapeutic interventions. Genomic DNA was separated from the samples using the QIAamp DNA Mini Kit (QIAGEN, Germany). The DNA concentration was measured using spectrometry. To sequence the mtDNA in the D-loop area, two intersecting partitions were amplified to cover the entire D-loop area by the polymerase chain reaction (PCR) method. The primers used are presented in Table 1. The master mix was prepared using 20 µl of the extracted DNA sample, 2 µl 10X PCR buffer (Mg<sup>2+</sup>, 20 mM), 0.5 µl sense and antisense primers, each equal to 10 µM, 0.2 µl polymerase (1.5 U/µl) derived by TransStart Taq (Beijing Transgen Biotech Co.), 1 µl DNA (20-50 ng/µl), and double-distilled water (16). During PCR, the samples were first denatured at 95°C for 5 min, followed by 40 cycles of denaturation at 95°C for 45 s and annealing at 58°C for 30 s. The reaction was completed with an extension step performed at 72°C for 45 s, with a final extension for 5 min. The PCR product was cleaned using the SAP mix (Thermo Fisher Scientific, Inc.), followed by cycle sequencing using 10

**Table 1.** Primers used to sequence the D-loop region.

PCR primers (5' - 3')	Sequence	Length
Mit23, F	TCATTGGACAAGTAGCATCC	756
Mit23, R	GAGTGGTTAATAGGGTGATAG	
Mit24, F	CACCATCCTCCGTGAAATCA	954
Mit24, R	AGGCTAAGCGTTTTGAGCTG	

µl of BigDye® Terminator v3.1 kit. The sense/antisense primers were used as a sequencing base for both forward and reverse sequencing. PCR and cycle sequencing were done in the PCR System 2720 (Thermo Fisher Scientific, Inc). The final product of the cycle sequencing was cleaned using the Big DyeX Terminator Purification kit. Afterward, capillary electrophoresis was performed using an ABI 3130 automated DNA tester (Thermo Fisher Scientific, Inc.). The sequencing outcomes were read and decoded by Chromas 2.23 (Technelysium Pty Ltd.). The Lasergene-Seqman program v.7.0 was used to interbreed four partitions in every sample with reference to the updated Cambridge guidelines on sequencing (NC\_012920.1). According to the guidelines, DNA sequences (mt16024-mt16569 and mt1-mt576) were produced for the full D-loop range. The DNA sequences were systematized per the revised Cambridge guidelines using CodonCode Aligner application v.4.0.4 to compare the outcomes.

### Histopathological Tests

All patients with thyroid cancer participating in the study underwent thyroidectomy. Samples from thyroid biopsy were obtained and examined by a pathologist. Histological evaluations were done on typical sections stained with hematoxylin and eosin as described. The tissue samples obtained from the biopsy were immediately covered in a 10%-solution of neutral buffered formalin (Sigma-Aldrich) for 8 h at room temperature. Tissue dehydration was ensured by gradually elevating alcohol degrees; following that, the tissues were cleaned using xylene. The tissues were then put in separate paraffin pads, and approximately 3-5µ sections were cut for routine histopathology analysis. For observation, the paraffin pads with the tissue sections were fixed on microscopic slides and stained with eosin and hematoxylin. The histopathological sections were analyzed and imaged using a specialized photomicroscope.

### Statistical Evaluation

All data were presented as mean ± standard deviation for continuous values and frequencies (%) for categorical values. Analysis was done using SigmaStat software v.3.5. Variations in frequency distributions between the groups were compared using the PLINK program v.1.07 by employing the exception rule to multiple alleles loci and mitochondrial microsatellite instability (mtMSI) deviations (D310, mt514-523 (CA)<sub>n</sub> and T16189C). SPSS was used to determine any association between mitochondrial deviations and clinical parameters. The statistical significance of the qualitative datasets was determined by a one-way ANOVA test, and that of the quantitative datasets was done using the unpaired t-test/Mann-Whitney U test. The chi-square test was used to determine if there were significant differences between the groups.

### Ethics Statement

This study was approved by the Deanship of Scientific Research for Princess Nourah Bint Abdulrahman University and sanctioned by the National Committee of Ethics (KACST, Saudi Arabia). All study participants were informed of the voluntary nature of their participation, and written consent was obtained prior to sample collection.

### Results

The age of the participants, both patients and the controls, ranged from 26 to 42 years. The demographic and pathological findings are summarized in Table 2. As compared to the controls, the proportion of patients aged 35 years and older was higher among the patients with PTC. The mean weight of the patients ranged between 61 and 70 kg. The mean age of participants in Group 1 (controls) was significantly lower than those in Groups 2, 3, and 4 ( $P < 0.0001$ ) (Table 2).

**Table 2.** Demographic and pathological findings of the Saudi women with PTC compared with that of the controls.

Covariates	Group 1:	Group 2:	Group 3:	Group 4:
	Controls	PTC + NV Diet	PTC + V diet	PTC + V diet + I
No. of participant	100	137	119	142
<b>Age (year)</b>				
Mean ± SD	33 ± 6.23	38 ± 2.74***	39 ± 3.82***	41 ± 2.31***
<b>Weight (kg)</b>				
Mean ± SD	64.6 ± 8.32	69.7 ± 6.41***	61.1 ± 11.03*	62.6 ± 5.24
<b>Number of participants with dietary iodine intake, n (%)</b>				
Natural (Dairy food)	94 (94%)	114 (83.21%)	0 (0%)	142 (100%)
Supplements	69 (69%)	34 (24.81%)	17 (14.3%)	136 (95.8%)
<b>Urinary iodine excretion (µg/l)</b>				
Mean (SD)	158.49 ± 9.56	143.21 ± 8.27***	14.12 ± 6.58***	187.6 ± 8.47***
<b>Tumor size, n (%)</b>				
1-3 cm	0	76 (55.5%)***	23 (19.3%)***	53 (37.3%)***
> 3 cm	0	61 (44.5%)***	96 (80.7%)***	89 (62.7%)***
<b>Histologic grade, n (%)</b>				
Low grade	0	78 (56.9%)***	35 (29.4%)***	81 (57%)***
High grade	0	59 (43.1%)***	84 (70.6%)***	61 (43%)***

P-values are in parentheses, \* $P < 0.05$ , \*\* $P < 0.001$ , \*\*\* $P < 0.0001$ . Papillary thyroid carcinoma (PTC), PTC patients following a non-vegetarian diet (Group 2: PTC + NV diet), PTC patients with standard vegetarian eating habits (Group 3: PTC + V diet), and PTC patients following a vegetarian diet and taking iodine supplements (Group 4: PTC + V diet + I).

The tumor sizes of the patients in Group 3 were significantly larger than those in Groups 2 and 4 ( $P < 0.0001$ ) (Table 2).

The proportion of participants with dietary iodine intake by natural means was nil among participants following a vegetarian diet (Group 3); 14.3% in this group were taking iodine supplements (Table 2). Accordingly, the mean urinary iodine excretion was the lowest in this group.

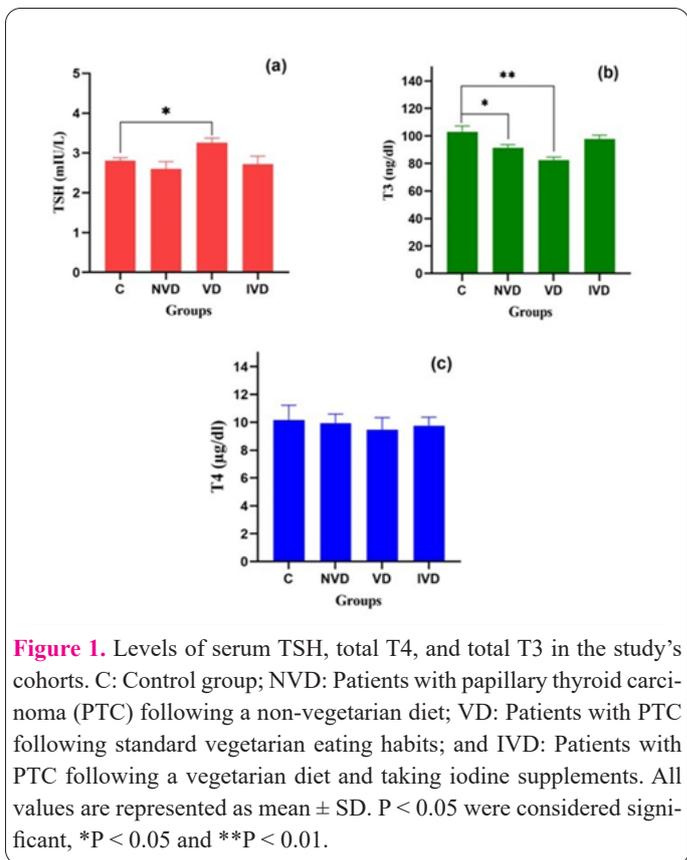
There were significantly higher percentages of patients with histologically high-grade tumors in Group 3 than in Groups 2 and 4 ( $P < 0.0001$ ) (Table 2). There was no significant difference between Group 2 and Group 4 in the proportion of patients with histologically high-grade tumors ( $P = 0.8$ ). High-grade histological features were more common in tumors with a size greater than 3 cm as compared to smaller-sized tumors.

**Thyroid profiles**

Mean TSH level was significantly higher in patients following a vegetarian diet (Group 3) than in controls (Group 1) with  $P < 0.05$ , whereas mean TSH levels in patients with non-vegetarian diets (Group 2) and iodine-supported vegetarian diets (Group 4) were similar to that in controls (Figure 1, a). Likewise, the mean total T3 level was significantly lower in patients following a non-vegetarian diet (Group 2) with  $P < 0.05$  and patients with a vegetarian diet (Group 3) with  $P < 0.01$  as compared to controls. In contrast, the mean total T3 in patients supplemented with iodine in a vegetarian diet (Group 4) was not significantly different from that in the controls (Figure 1, b). Nonetheless, no significant associations were found in total T4 levels between the groups (Figure 1, c).

**Frequency of mtDNA mutations in the D-loop region**

The distribution of identified mutations in the whole mtDNA D-loop area among all participants is provided in Figure 2 and Table 3. None of the controls had any mtDNA mutations, while the patients with PTC showed an increased frequency of mtDNA mutations in the D-loop region. The mutations were identified and marked as somatic (physical) mutations or germline mutations (Figure 2 and Table



**Figure 1.** Levels of serum TSH, total T4, and total T3 in the study's cohorts. C: Control group; NVD: Patients with papillary thyroid carcinoma (PTC) following a non-vegetarian diet; VD: Patients with PTC following standard vegetarian eating habits; and IVD: Patients with PTC following a vegetarian diet and taking iodine supplements. All values are represented as mean  $\pm$  SD.  $P < 0.05$  were considered significant, \* $P < 0.05$  and \*\* $P < 0.01$ .

3). The frequencies of mtDNA D-loop mutations and the types of mutations (somatic or germline) were significantly different between all the study groups (Table 3). While most mutations identified in Group 3 were somatic mutations, germline mutations were more common in Groups 2 and 4. More specifically, mtDNA mutations, such as A164G, T172C, A182C, A183C, T189C, C223T, T304C, and T519C repeats, were due to inclusions or deletions at unstable regions. It was revealed that the frequencies of T189C (28.57%) and A164G (23.52%) somatic mutations were significantly high in patients with PTC following a vegetarian diet. The T304C germline mutation was significantly higher in patients with PTC following a non-vegetarian diet (30.65%, 42/137) and in patients following

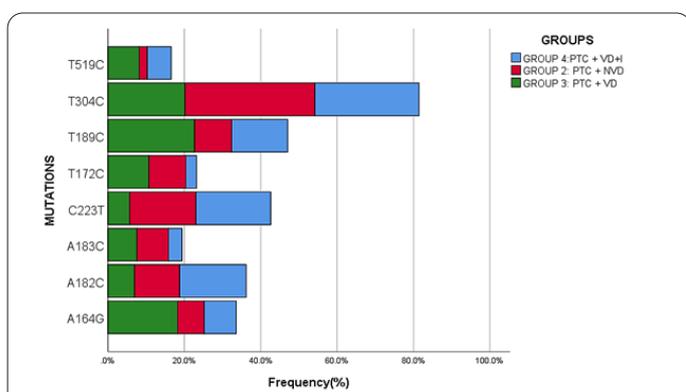
**Table 3.** Distribution of mtDNA D-loop mutations in the study groups; Group 1 (n=100), Group 2 (n=137), Group 3 (n=119), and Group 4 (n=142).

Mutations		Somatic or germline: n (%)				Reported	P-values
Position	Change	Group 1: Controls	Group 2: PTC +NVD	Group 3: PTC + VD	Group 4: PTC + VD + I		
16164	A-G	0 (0.00%)	S: 2 (1.45%) G: 8 (5.83%)	S: 28 (23.52%) G: 1 (0.84%)	S: 0 (0.00%) G: 12 (8.45%)	Yes	0.0001
16172	T-C	0 (0.00%)	S: 3 (2.18%) G: 11 (8.02%)	S: 17 (14.28%) G: 0 (0.00%)	S: 0 (0.00%) G: 4 (2.81%)	Yes	0.0001
16182	A-C	0 (0.00%)	S: 1 (0.72%) G: 16 (11.68%)	S: 10 (8.40%) G: 1 (0.84%)	S: 6 (4.22%) G: 19 (6.33%)	Yes	0.0001
16183	A-C	0 (0.00%)	S: 8 (5.83%) G: 4 (2.92%)	S: 11 (9.24%) G: 1 (0.84%)	S: 0 (0.00%) G: 5 (3.52%)	Yes	0.0001
16189	T-C	0 (0.00%)	S: 0 (0.00%) G: 14 (10.21%)	S: 34 (28.57%) G: 2 (1.68%)	S: 4 (2.81%) G: 17 (11.97%)	Yes	0.0001
16223	C-T	0 (0.00%)	S: 0 (0.00%) G: 25 (18.25%)	S: 9 (7.56%) G: 0 (0.00%)	S: 1 (0.70%) G: 27 (19.01%)	Yes	0.0001
16304	T-C	0 (0.00%)	S: 7 (5.10%) G: 42 (30.65%)	S: 27 (22.68%) G: 5 (4.20%)	S: 3 (2.11%) G: 36 (25.35%)	Yes	0.0001
16519	T-C	0 (0.00%)	S: 0 (0.00%) G: 3 (2.18%)	S: 13 (10.92%) G: 0 (0.00%)	S: 0 (0.00%) G: 9 (6.33%)	Yes	0.0001

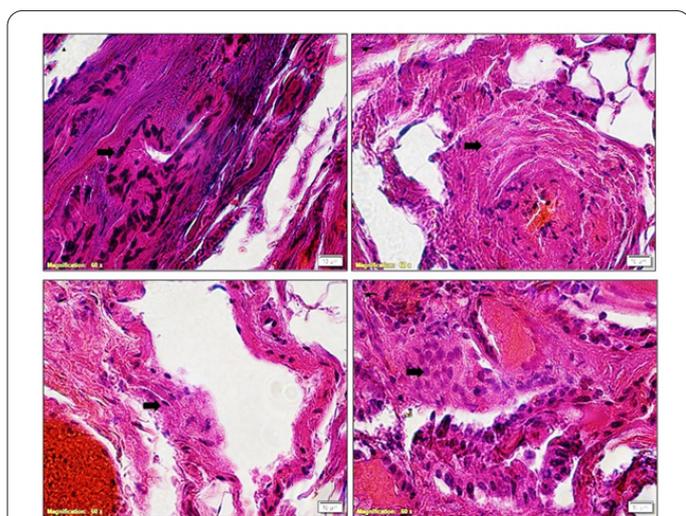
iodine-supported vegetarian diets (25.35%, 36/142).

### Histopathological outcomes

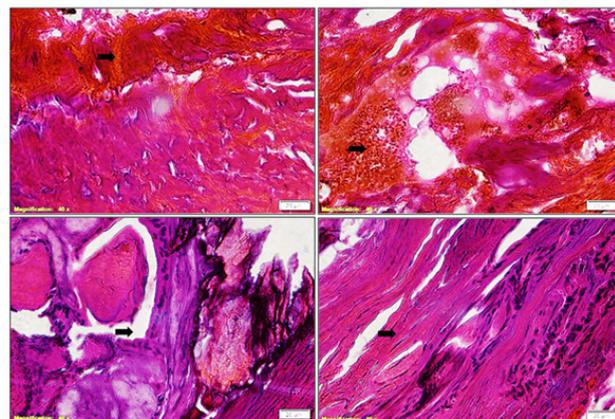
Histopathological findings of the participants with PTC are shown in Figures 3-5. All observations indicated a dispersed correlation with at least one thyroid lobe, along with potential fibrosis, frequent psammoma bodies, squamous metaplasia, and regular lymphatic intervention. The carcinoma cells demonstrated carcinogenic patterns; for example, neoplasms were aligned as macro- or micro-follicles; they had central colloid and squamous morules, incorporating randomly directed papillae as well as fibrovascular cores. Nuclear alterations in size, such as nuclear increase and prolongation, and alterations in forms, such as columnar/cigar-shaped nuclei (demonstrating visible supra/subnuclear vacuoles) were also identified. The histological findings of patients in Group 3 demonstrated advanced carcinomatosis patterns, destructed cell structures



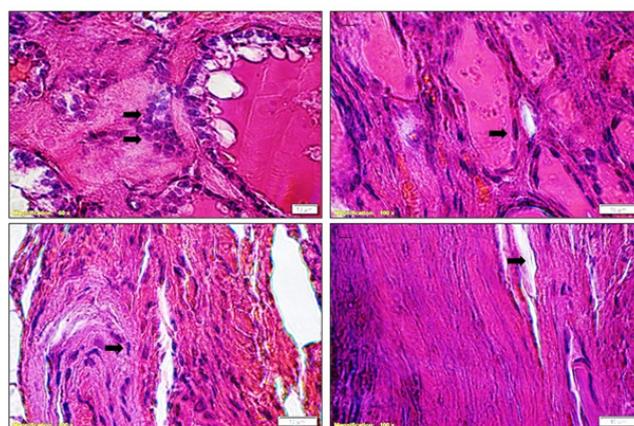
**Figure 2.** Frequency of mtDNA mutations in the D-loop area among all cohorts (%). All the mutations of mtDNA were recorded in the study groups as follows: Patients with papillary thyroid carcinoma (PTC) following a non-vegetarian diet (Group 2: PTC + NVD), patients with PTC with standard vegetarian eating habits (Group 3: PTC + VD), and patients with PTC following a vegetarian diet and taking iodine supplements (Group 4: PTC + VD + I).



**Figure 3.** Photomicroscopic images of (hematoxylin and eosin) stained samples taken from participants in group 2: patients with PTC following a non-vegetarian diet. The histopathological sections revealed dispersed tissue with patterns of carcinomatosis, such as nuclear alterations in size and shape (columnar/cigar-shaped nuclei), destructed cell structures, tissues filled with RBCs (clusters of red cells) and necrosis (indicated by the spaces in the body tissues).



**Figure 4.** Photomicroscopic images of (hematoxylin and eosin) stained samples taken from participants in group 3: patients with PTC following a vegetarian diet. The histological sections showed advanced carcinomatosis patterns, highly destructed cell structures filled with RBCs (clusters of red cells), and an increased share of damage in thyroid tissues.



**Figure 5.** Photomicroscopic images of (hematoxylin and eosin) stained samples taken from participants in group 4: patients with PTC following a vegetarian diet with iodine supplement. The histopathological sections revealed minor carcinomatosis patterns, such as slight nuclear alterations in size and shape (columnar/cigar-shaped nuclei) and destructed cell structures and tissues.

filled with red blood cells (RBCs), and an increased share of damage in thyroid tissues.

### Discussion

In this study, blood samples from females diagnosed with PTC in Saudi Arabia were tested and analyzed for potential mutations in mtDNA. The diet of these patients was also analyzed to examine possible associations between iodine deficiency, mtDNA mutations, and PTC. More somatic mutations were observed in patients with PTC following a vegetarian diet (Group 3) as compared to patients following a non-vegetarian diet (Group 2) or a vegetarian diet with an iodine supplement (Group 4).

Several earlier studies have reported that insufficient or low consumption of iodine leads to elevated TSH, increasing the risks of thyroid cancer development as well as association with increased tumor size, more severe histological features, and tumor progression (17-22). In addition, the results of some studies are indicative of iodine deficiency in people on a vegetarian or vegan diet

without any supplements (23-25). Similarly, in our study, the mean urinary iodine levels ( $\mu\text{g/l}$ ) were significantly lower in patients with PTC following vegetarian diets than in other PTC groups and controls. Accordingly, the mean TSH level was significantly higher ( $P < 0.05$ ) among patients with PTC following a vegetarian diet. A significantly higher proportion of patients in this group of patients had a tumor size of  $> 3$  cm and higher degrees of histopathological findings. On the other hand, patients following a non-vegetarian or vegetarian diet with iodine supplements showed TSH levels similar to controls. The total T3 levels were significantly lower in patients with PTC following non-vegetarian ( $P < 0.05$ ) and vegetarian ( $P < 0.01$ ) diets as compared to controls, while these levels were similar in patients with PTC following a vegetarian diet with iodine supplement and in controls. No significant changes were observed in total T4 levels between groups. Similar observations were reported by Tognini et al., with a significant correlation between decreased T3 levels and metastasized carcinoma among elderly in-patients ( $P = 0.0002$ ) (26). Another study reported non-significant changes in T3 and T4 levels in patients with PTC (27). In general, epidemiological studies exploring the relationship between iodine consumption and thyroid cancer present mixed and equivocal findings.

More recently, mtDNA mutations and changes in the D-loop area have been identified as driving factors in carcinogenesis (28, 29). In this study, somatic and germline mutations were identified, and somatic mutations were predominant among patients with PTC following a vegetarian diet. The somatic deviations were predominantly  $T \rightarrow C$  and  $A \rightarrow G$  and germline deviations were mainly  $T \rightarrow C$  transitions and indicate a possibility of mtMSI. Higher PTC development and pathogenesis rates were reported upon sequencing the mtDNA within the D-loop area of 66 PTC patients, throwing insights into molecular patterns involved in PTC genesis (30). In another study by Abu-Amero et al., 36.8% of samples from 19 patients with PTC had somatic mutations, with most mutations occurring in the mitochondrial complex I genes (31). Based on the findings of another study, the pathogenicity score of somatic mtDNA mutations and variations in mitochondrial complex I could be potential determinants of the oncogenic phenotype of cancer cells (32). The mtDNA mutations are considered a critical molecular driver of the mtMSI in various neoplasms, contributing to cancer development and tumorigenesis (33, 34). These data indicate the involvement of mtDNA mutations in early tumorigenesis, and the findings may be instrumental in developing potential biomarkers or therapeutic targets for more efficient clinical interventions.

The reason for the female predominance of differentiated thyroid cancer is not fully understood. However, one possible explanation could be disturbances in the levels of thyroid hormones during pregnancy and postpartum (35, 36). Reportedly, iodine deficiency is a risk factor for thyroid cancer, in addition to family history, obesity, radiation impact, and increased leptin. There have been some reports on recent trends and increases in preferences for vegetarian or vegan diets and eating disorders among young adolescents and females in Saudi Arabia (37). Dietary restrictions could also lead to the deficiency of other micronutrients, such as selenium, iron, and zinc, that may play critical roles in thyroid functions (6). However, well-

planned vegetarian diets with supplements were found to fulfill micronutrient requirements in earlier studies (38). Likewise, in our study, the thyroid hormone profile and mtDNA mutations of the group following a vegetarian diet supplemented with iodine were comparable with that of the group following a non-vegetarian diet. Inheritance of mtDNA mutations has also been found to be associated with cancer risk (39-41), while the dynamics and progression of the disease could be influenced by environmental and lifestyle factors (42, 43). Thus, genetic, environmental, and lifestyle factors can affect the development and progression of PTC (44).

### Conclusion

This is the first complex study involving the analysis of dietary intake and its impact on iodine status, mtDNA mutations, and PTC development among Saudi women. Our results indicated a possible association between iodine deficiency and accelerated pathogenesis and tumorigenesis by amplified replication of cancer cell structures, evidenced by the somatic mutations in mtDNA in patients with PTC following a vegetarian diet. These genetic mutations can serve as diagnostic biological markers and potential therapeutic targets. However, further studies with a larger sample size are recommended to validate and extend the current findings to other population groups.

### Declaration

#### Competing interests

The authors do not have anything to disclose and declare no conflicts of interest.

#### Ethics Statement

The study was approved by the Deanship of Scientific Research for Princess Nourah Bint Abdulrahman University and sanctioned by the National Committee of Ethics (KACST, Saudi Arabia). The study participants were females, either healthy (control group) or diagnosed with papillary thyroid cancer (cases), and were recruited in collaboration with KFMC between August 2020 and November 2020. All participants were informed of the voluntary nature of their participation, and written consent was obtained prior to sample collection. Updated guidelines complied during the implementation stages of the study were followed throughout the study.

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#### Conflicts of interest

The authors have declared that no competing interests exist.

#### Data availability statement

The data that support the findings of this study are avail-

lable from the corresponding author, upon reasonable request.

### Authors' contributions

M.A.B. Methodology, Software data curation, Visualization, Investigation, Reviewing and editing the manuscript. W.H.A. Methodology, Investigation, Reviewing and editing the manuscript. M.A.A. Software data curation, Investigation, Reviewing and editing the manuscript. S.M.S.A. Software data curation, Visualization, Reviewing and editing the manuscript. H.S.A. Methodology, Investigation, Reviewing and editing the manuscript. D.M.D. Methodology, Software data curation, Reviewing and editing the manuscript. F.A.S. Methodology, Visualization, Investigation, Reviewing and editing the manuscript. M.A.A. Methodology, Software data curation, Reviewing and editing the manuscript. N.A.A. Methodology, Investigation, Reviewing and editing the manuscript. M.A.A. Methodology, Investigation, Software data curation, Reviewing and editing the manuscript. W.S.A. Designing the study, Conceptualization, Methodology, Performing the statistical analysis, Software data curation, Visualization, Investigation, Writing the original draft, and Submitting the manuscript as a corresponding author.

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