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Diet and Lifestyle Role in Homocysteine Metabolism in Turner Syndrome

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Significance of the Study

- Patients with Turner syndrome have an unfavorable cardiometabolic profile. In this study, we show that an inadequate plasma homocysteine and vitamin B_{12} level was present in Turner syndrome, even though vitamin supplementation was prescribed. Diet, lifestyle and therapies affect the metabolism of homocysteine; no influence of the genetic *MTHFR* profile on homocysteine levels was noted. Educational approaches to a healthy lifestyle can reduce the cardiovascular risk of these patients.

Keywords
Diet · Lifestyle · Homocysteine · Turner syndrome · Cardiovascular risk

Abstract
Objective: Patients with Turner syndrome (TS) have an unfavorable cardiometabolic profile. Hyperhomocysteinemia is a potential cardiovascular risk factor influenced by genetic and environmental factors, therapies, unbalanced diets and other lifestyle factors. We retrospectively studied the relationship between total plasma homocysteine (Hcy), serum vitamin B_{12} (B_{12}) and folate concentration in TS patients, taking into account the genetic profile, diet, smoking habits, hormonal therapies and dietary supplements of the subjects. ***Patients and Methods:*** We evaluated 50 TS patients (31.5 ± 12.5 years). Medication, including vitamin supplementation, was obtained. Eating habits, cigarette smoking, alcohol and coffee consumption were investigated using phone interviews. Levels of Hcy metabolism parameters were classified by using the relevant cutoff value for an adult population and compared with a reference sample drawn from the general population. ***Results:*** Inadequate Hcy and B_{12} levels were noted, despite vitamin supplementation. Holotranscobalamin (HoloTC) was above the relevant cutoff in the population, and supplemented subjects showed mean levels lower than nonsupplemented subjects ($p = 0.005$). Dietary supplementation ($p = 0.038$), lifestyle (coffee consumption, $p = 0.01$) and hormonal replacement therapy ($p = 0.02$) are important factors for Hcy metabolism. No genetic influ-

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ence on Hcy levels was noted. Multivariable regression analysis identified vitamin supplementation ($p = 0.045$) as the only independent predictor of increased Hcy levels. ***Conclusion:*** Cardiovascular risk in TS can be reduced using educational approaches to a healthy lifestyle with dietary guidelines. Besides this, we also recommend measuring HoloTC for the prompt detection of B_{12} deficiency and to consider hormone replacement therapy in the biochemical assessment of homocysteine in TS.

Introduction

Turner syndrome (TS) is a genetic condition caused by complete or partial absence of an X chromosome. It is the most commonly diagnosed sex chromosome abnormality in women, affecting 1/2,000–2,500 female live births and is usually associated with retarded growth, reduced adult height and gonadal dysgenesis [1, 2]. Growth hormone (GH) treatment is often given during infancy to increase attained height [3, 4], and hormonal replacement therapy (HRT) is prescribed to initiate and sustain sexual maturation [1, 3].

Young women and girls with TS have unfavorable cardiometabolic risk factors, such as congenital cardiovascular structural abnormalities, hypertension, low birth weight, increased prevalence of obesity, frequent glucose intolerance and dyslipidemia which predispose them to adverse cardiac outcomes earlier than in the general population [5]. Additional risk factors, such as elevated plasma total homocysteine levels (tHcy) have also been described in TS patients [6]

Homocysteine (Hcy) is a sulfur amino acid, and its metabolism consists of the intersection of two pathways: remethylation to methionine, which requires folate, vitamin B_{12} (B_{12}) coenzymes and trans-sulfuration to cysteine, which requires vitamin B_6 coenzyme [7]. Increased levels of plasma tHcy lead to a hyperhomocysteinemia which is considered as a potential risk factor for cardiovascular diseases, venous thrombosis, vascular complications and systemic disorders [8].

Among the causes of hyperhomocysteinemia there are genetic and environmental factors, various diseases, pharmacological therapies, unbalanced diets and other lifestyle factors [9]. Particularly, increased levels of plasma tHcy may be a result of the genetic defects of enzymes involved in its metabolism, such as cystathionine-β-synthase, methionine synthase and methylenetetrahydrofolate reductase *(MTHFR)*. Moreover, smoking habits, alcohol and caffeine abuse, and a diet low in fruits and vegetables, are further causes of hyperhomocysteinemia [9].

Deficiency of vitamins B_2, B_6, B_{12} and folate, due to inadequate dietary intake or gastrointestinal malabsorption, are acknowledged. Additionally, analysis of the literature reveals an increased incidence of gastrointestinal autoimmune diseases, in particular chronic inflammatory bowel diseases (2.6%) and celiac disease [10, 11] that may contribute to such nutritional deficiencies; however, nowadays nutritional status has been poorly investigated in TS.

Based on these considerations, we conducted a retrospective observational study on patients with TS, with the objective of studying the relationship between plasma Hcy, serum B_{12} and folic acid levels, taking into account genetic profiles, diet, smoking habits, hormonal therapies and dietary supplements.

Patients and Methods

Patients

We evaluated 50 TS patients (mean age at evaluation 31.5 ± 12.5 years) who attended the Pediatric Endocrinological Unit of the Department of Maternal and Children's Health, Fondazione IRCCS Policlinico San Matteo, Pavia, Italy

Karyotype analysis showed 45,X in 31 patients, X-mosaicism and/or structurally abnormal X chromosome in 19 patients; in 3 of 45,X patients the presence of an occult Y fragment was present. Eighteen (36%) TS subjects presented with associated autoimmune diseases (Table 1): 36 girls (72%) were treated with GH, 35 (70%) with HRT and 11 (22%) with L-thyroxine. Clinical as well as genetic features and treatment of patients are reported in Table 1.

For every patient, plasma Hcy, serum folate (s-F), B_{12} and holotranscobalamin (HoloTC) levels were measured in order to evaluate the Hcy metabolism. Additionally, C677T and A1298C polymorphisms in *MTHFR* were also investigated. Levels of Hcy metabolism parameters were classified by using the relevant cutoff value for an adult population and were compared with a reference sample group drawn from the general population, matched for age and gender [12–15].

In all patients, assessments of medication, including vitamin supplementation, were obtained. Evaluations of eating and lifestyle habits, including cigarette smoking, alcohol and coffee consumption, were investigated by phone interviews of skilled dieticians.

All patients were provided with a consent form at the time of their visit addressing privacy and confidentiality and were invited to sign an agreement authorizing the use of their medical records for research purposes. Ninety-two percent of patients authorized the use of their medical records for this purpose. The study was performed according to the Declaration of Helsinki; Institutional Review Board approval for retrospective data collection was obtained per institutional guidelines.

Table 1. Clinical characteristics and patient treatments, according to karyotype

Characteristics	Karyotype		
	total (n = 50)	45,X (n = 31)	non-45,X (n = 19)
Mean age at TS diagnosis, years	7.56±5.44	7.74±5.40	7.77±5.90
Mean age at evaluation, years	31.55±12.54	32.74±13.1	29.61±11.53
Autoimmune diseases, *n*			
Thyroiditis	15 (30%)	11 (22%)	4 (8%)
Celiac disease	3 (6%)	1 (2%)	2 (4%)
Crohn's disease	1 (2%)	1 (2%)	0 (0%)
Atrophic gastritis	2 (4%)	1 (2%)	1 (2%)
Treatment, *n*			
Growth hormone	36 (72%)	21 (42%)	15 (30%)
Estroprogestin	35 (70%)	23 (46%)	12 (24%)
Thyroxine	11 (22%)	7 (14%)	4 (8%)

Methods

Biochemical Parameters

Blood samples were drawn in the morning, after an overnight fast. Two blood specimens from each patient were collected in light-protected tubes, either with no additive (for serum B_{12}, HoloTC, s-F) or containing ethylenediaminetetraacetic acid (EDTA) to prevent coagulation, for tHcy concentration assays. EDTA specimens were immediately put on ice and were centrifuged within 30 min in order to obtain plasma samples for tHcy determination. Serum and plasma samples were frozen and stored at –80 °C until analysis.

Plasma tHcy levels were measured by a competitive immunoassay using direct chemiluminescence technology. The different forms of Hcy in the patient sample are reduced to free Hcy by the reducing reagent; free Hcy is then converted to S-adenosylhomocysteine and measured by a specific immunoassay (Advia Centaur, Siemens Healthcare Diagnostics). Hyperhomocysteinemia was defined for plasma tHcy levels >10.0 μmol/L as previously described by Zappacosta et al. [12].

Levels of Hcy metabolism parameters were classified by using the relevant cutoff values for an adult population [12–15].

Serum B_{12} was measured by a solid-phase, competitive chemiluminescence enzyme immunoassay involving an automated alkaline denaturation procedure (Immulite 2000, Siemens Healthcare Diagnostics, Llanberis, UK). s-F concentrations were determined by a competitive immunoassay based on a 2-cycle on-board sample treatment of patient serum (Immulite 2000, Siemens Healthcare Diagnostics, Llanberis, UK). Cutoff values for adequate B_{12} and s-F levels were used according to Dhonukshe-Rutten et al. [13] and Daly et al. [14], respectively, for cardiovascular disease and neural tube defects prevention, that do not necessarily reflect those for nutritional deficiency.

HoloTC was measured by an immunoenzymatic assay using the relevant commercial kits on an Architect analyzer i2000SR (Abbott Diagnostics, Abbott Park, IL, USA). HoloTC levels were classified by using the relevant cutoff value for an adult population [15].

MTHFR C677T and A1298C were tested by reverse hybridization, where specific oligonucleotide probes immobilized as parallel lines on membrane-based strips hybridize with biotinylated PCR products (Nuclear Laser Medicine, Settala, Italy).

Diet, Lifestyle, Dietary Supplementation

Concerning the eating habits, we examined the daily consumption of raw leafy vegetables and bi- and triweekly meat and/or fish; responses were classified into "0" (no consumption) or "1" (consumption). Daily consumption of coffee and alcohol was also assessed by specific questions on daily frequency and quantity during the phone interview conducted by the dietitian. As regards smoking habits, patients were classified as "never," "current" or "former" smokers. All current smokers were asked to report the number of cigarettes smoked per day.

Finally, folic acid and/or B_{12} supplementation was assessed; variables were classified as "0" (not supplemented) or "1" (supplemented).

Statistical Analysis

Continuous data were described as means ± standard deviation and categorical data as counts and percent. Generalized linear regression models were used to compare levels of the different metabolites between groups of patients. Log transformation was used for vitamin B_{12} and folates. The mean difference and 95% confidence intervals were retrieved from the model. Given the low number of subjects, an exploratory multivariable regression model for tHcy was fitted including *MTHFR* mutation and predictors with $p < 0.1$ at univariable analysis. A p value <0.05 was considered statistically significant. Bonferroni correction was applied for post hoc comparisons. Stata 14.2 (StataCorp, College Station, TX, USA) was used for computation.

Results

Diet, Lifestyle, Dietary Supplementation

With regard to diet and lifestyle, we collected data from 37 TS patients. Daily consumption of raw leafy veg-

Table 2. Biochemical parameters of TS patients

Analytes (adequate *cutoff* or reference interval)	Mean values (SD)	Altered levels, %
Total plasma homocysteine (<10.0 nmol/L)	11.2 (3.1)	82
Vitamin B_{12} (>470 pg/mL)	384.6 (170.4)	73.5
Holotranscobalamin (>40 pmol/L)	90.1 (29.7)	2
Serum folate (>6.6 ng/mL)	9.7 (6.7)	44.9
Hemoglobin (12–16 mg/dL)	13.5 (0.8)	0
Mean corpuscular volume (80–96 fL)	92.3 (4.4)	0

Data are reported as means with standard deviations (SD) in parentheses. Percentages of altered values are also reported.

etables and the consumption of meat and/or fish, at least 3 times a week, was recorded in 43.2% (n = 16) and 78.4% (n = 29) of the subjects, respectively.

About 65% (n = 24) consumed at least 1 cup of coffee per day; in particular, 40.5% (n = 15) consumed ≤2 cups of coffee/day; 24.5% (n = 9) consumed >2 cups of coffee/day. One alcoholic unit per day was consumed by 5.4% of patients (n = 2). As far as smoking is concerned, 86.5% (n = 32) of TS patients were nonsmokers, 8.1% (n = 3, mean number of cigarettes smoked/day = 4) were current smokers and 5.4% (n = 2) were former smokers.

Concerning B_{12} and/or folic acid supplementation, 32% of patients (16/50) were supplemented. In particular, 2% (n = 1) received only B_{12} supplementation; 18.4% (n = 9) received only folic acid supplementation; 12% (n = 6) received both B_{12} and folic acid supplementation.

MTHFR Gene Polymorphism C677T, A1298C

Mutation in the *MTHFR* gene was present in 37 (74%) girls, with 29 patients (58%) being heterozygous and 8 (16%) homozygous. The *MTHFR* C677T allele was present in 27 subjects (77.1%), the A1298C allele in 4 girls (11.4%) and both C677T and A1298C in 4 patients (11.4%).

Biochemical Parameters

Biochemical parameters of TS patients are listed in Tables 2 and 3.

Hematological Status

All subjects had an adequate hematological status, based on a standard complete blood count panel [16], and normal renal function (data not reported), as shown in Table 2.

Hcy and s-F

Most of the sample (82%) reported tHcy levels higher than a cutoff level [12], and mean tHcy levels indicated a mild hyperhomocysteinemia condition (Table 2).

Interestingly, supplemented patients reported significantly higher tHcy levels than nonsupplemented ones (p = 0.038), showing a mild hyperhomocysteinemia (Table 3). Moreover, tHcy levels did not differ significantly between subjects supplemented only with folic acid and subjects supplemented with both folic acid and B_{12} (13.9 ± 2.6 vs. 10.5 ± 2.9 μmol/L, p = 0.034).

The evaluation of the Hcy metabolically related vitamins highlighted mean s-F levels above the adequate cutoff value [14] (Table 2); however, low values were observed in 44.9% of the sample [14] and significantly higher values in supplemented subjects than in nonsupplemented ones (p = 0.009) (Table 2).

Plasma tHcy levels did not differ significantly between patients with or without *MTHFR* mutation (11.4 ± 2.39 and 11.1 ± 3.4, respectively, p = 0.7). *MTHFR* tHcy levels were not significantly different in patients with homozygous and heterozygous mutations (10.9 ± 3.2 and 12.1 ± 4.1, respectively, p = 1) and according to C677T, A1298C and C677T + A1298C alleles (11.2 ± 3.6, 9.9 ± 2.1 and 11.5 ± 3.5, respectively, p = 0.75). s-F levels were not influenced by *MTHFR* mutation and daily consumption of raw leafy vegetables, but folic acid supplementation (p < 0.001) was found to be an independent factor for the increase in levels of s-F.

Concerning HRT, tHcy levels were significantly decreased and under the cutoff level [12] in patients without HRT than in patients with HRT (9.02 ± 2.8 vs. 12.2 ± 2.8 μmol/L; p = 0.001), with a positive association between tHcy levels and HRT (p = 0.02).

Exploratory multivariable regression analysis identified vitamin supplementation (p = 0.045) as the only independent predictor of increased plasma tHcy levels (Table 4).

Table 3. Plasma total homocysteine (tHcy), serum folate (s-F), vitamin B_{12} (B_{12}) and holotranscobalamin (HoloTC) levels in TS patients

	tHcy (<10.0 nmol/L)	*p*	B_{12} (>470 pg/mL)	*p*	HoloTC (>40 pmol/L)	*p*	s-F (>6.6 ng/mL)	*p*
Dietary supplementation								
Supplemented	12.5 (3.0)	0.038	266.8 (52.0)	<0.001	73.5 (26.8)	0.006	14.5 (8.7)	0.012
Not supplemented	10.6 (3.0)		441.6 (178.8)		97.9 (28.1)		7.4 (3.9)	
Alcohol comsumption								
No comsumption	10.8 (2.8)	0.06	373.4 (174.5)	0.48	91.2 (31.3)	0.20	9.63 (6.6)	0.12
Almost 1/day	14.7 (0.64)		281 (76.4)		62.4 (4.3)		17.6 (9.0)	
Coffee consumption								
≤2/day	10.39 (2.8)	0.01	380 (184.4)	0.55	90.8 (32.7)	0.68	10.0 (6.5)	0.79
>2/day	12.97 (2.0)		331.5 (126.1)		85.8 (25.9)		10.1 (8.2)	
Smoking habit								
No smoker	10.6 (2.8)	0.06	371.9 (180.0)	0.91	88.1 (31.3)	0.44	10.1 (6.7)	0.71
Current or former smoker	13.3 (2.1)		345.6 (114.2)		99.6 (29.4)		9.4 (8.3)	
Raw leafy vegetables every day								
Yes	11.1 (3.3)	0.94	373.5 (157.8)	0.59	89.5 (29.8)	0.97	9.2 (6.3)	0.45
No	11.0 (2.1)		361.0 (194.2)		89.8 (33.3)		11.1 (7.6)	
Meat and/or fish at least 3 times per week								
Yes	11.0 (2.3)	0.97	312.8 (26.3)	0.57	83.3 (28.5)	0.52	8.1 (5.6)	0.46
No	11.4 (3.0)		381.7 (188.6)		91.4 (31.8)		10.5 (7.1)	

B_{12} and HoloTC

Mean levels of vitamin B_{12} were under the adequate cutoff values [13]. Low values were observed in 73.5% of the subjects and were significantly lower in supplemented than in nonsupplemented subjects ($p < 0.001$, respectively) (Tables 2, 3). HoloTC was under the relevant cutoff in only 1 patient [15], and supplemented subjects showed lower mean levels compared to those not supplemented ($p = 0.005$), even within the relevant cutoff value (Tables 2, 3).

Table 4. Multiple regression analysis between plasma tHcy levels and different influencing factors

Independent variables	Difference	95% CI	*p*
MTHFR mutation	0.20	–2.07 to 2.11	0.98
Supplementation	2.46	0.04 to 4.11	0.04
Coffee consumption	1.68	–0.45 to 3.83	0.11
Alcohol consumption	1.34	–2.829 to 5.52	0.51
Smoking habits	1.45	–1.24 to 4.14	0.28

Discussion

In this retrospective study, inadequate tHcy and B_{12} were noted in the TS population, even though vitamin supplementation was prescribed. Diet, lifestyle and therapies affect Hcy metabolism. No significant influence of the genetic *MTHFR* profile on tHcy levels was noted in TS.

Current epidemiological evidence suggests that young women and girls with TS have unfavorable cardiometabolic risk factors, which predispose them to adverse cardiac and cerebrovascular outcomes earlier than in the general population [5]. Dietary behavior is an important modifiable factor in cardiovascular disease prevention also in TS. Nutritional deficiencies, such as folate and vitamin B_{12} may lead to an increase in cardiovascular disease markers, such as Hcy. Hyperhomocysteinemia is well established as an independent risk factor for coronary heart disease and arteriosclerosis. Studies have shown that coronary heart disease is linked to plasma Hcy levels, with a substantial risk occurring at >10 μmol/L plasma Hcy; furthermore, each 5 μmol/L increase in plasma Hcy is associated with a 20% increased risk of coronary heart disease events [17]. The etiological factors for atherosclerosis raise conversion of methionine to Hcy thiolactone, which is the reactive cyclic internal lactone of Hcy, leading to a consequent thiolation reaction of amine-containing low-density lipoprotein. These reactions produce the aggregation and increased uptake of low-density lipoprotein by macrophages, explaining the observed phenomenon of lipid deposition in atheroma and atherosclerotic plaques [18–20] throughout intimal

injury, platelet aggregation, thrombogenic factors, fibrosis and calcification.

Levels of Hcy in tissues and plasma are influenced by many genetic and environmental factors, as well as certain medications and lifestyle. Change in tHcy levels may be caused by impaired renal function, more rarely by enzyme modification of activity and more frequently by low vitamin B intake [8], especially vitamin B_{12} and folate, although two other vitamins play an important role in Hcy metabolism: vitamin B_2, which serves as cofactor for the methyl-metabolizing enzymes methylenetetrahydrofolate reductase, regenerating 5-methyltetrahydrofolate from tetrahydrofolate, and methionine synthase reductase, which activates methionine synthase, and vitamin B_6, which is cofactor for the enzyme cystathionine ß-synthase involved in the trans-sulfuration pathway in which Hcy is converted into cystathionine.

In our TS population, we observed tHcy levels above the cutoff value in most of the subjects and inadequate levels of s-F and B_{12} in 44.9 and 73.5% of subjects, respectively. Of note, we referred to the cutoff values of Dhonukshe-Rutten et al. [13] for the adequate B_{12} and folate status for the prevention of cardiovascular disease, which do not necessarily reflect those for nutritional deficiencies.

MTHFR enzyme is required in the tHcy recycling phase. Inherited genetic mutations in the folate-related enzyme genes can affect its activity and, consequently, may lead to elevated homocysteine levels. Single-nucleotide polymorphisms in the enzyme are common, especially the two low-functioning variants: C677T and A1298C. In particular, the C677T variant is associated with an increased risk of thromboembolism in adults [21]. Homozygote (TT genotype) individuals produce a thermolabile enzyme, which results in a 40–50% decreased activity, affecting the remethylation step and causing mild hyperhomocysteinemia [21]. In our patients, tHCy levels did not differ among subjects with or without mutation, suggesting the importance of vitamin supplementation despite the genetic profile, as the involvement of chromosomal imbalances on *MTHFR* is not excluded [22].

It has been shown that B group vitamins have an important role in lowering tHcy levels because of their direct involvement in the Hcy remethylation pathway [23]. Meta-analyses of B vitamin supplementation trials consistently showed that ≥0.5 mg/day of folic acid dose-dependently reduced tHcy levels and that adding 0.4 mg/day vitamin B_{12} led to a further reduction of 7% [24].

In our study, about 33% of patients were supplemented with folic acid and/or B_{12}. Supplemented TS patients showed adequate mean s-F levels, significantly higher than nonsupplemented TS subjects, suggesting substantive adherence to the supplement prescription. On the contrary, B_{12} levels were significantly lower, and under the adequate cutoff value, in supplemented than in nonsupplemented TS subjects. Additionally, in contrast to previous findings [24], tHcy levels did not differ significantly between patients supplemented with folic acid and patients supplemented with folic acid and B_{12}. This could be related to poor adherence to dietary supplements, of which health care professionals may be unaware.

Analyzing cobalamin status, HoloTC levels were above the cutoff value in the whole study population. Although supplemented TS subjects showed significantly lower HoloTC levels compared to nonsupplemented TS patients, both groups reported adequate HoloTC levels suggesting that TS patients were not at risk of B_{12} deficiency. In fact, HoloTC, the transcobalamin-cobalamin complex, represents the biologically active form of the vitamin and consists of 10–30% of total serum B_{12} [15]. This complex is recognized by ubiquitous specific membrane receptors and could have a high diagnostic value as a marker of storage [15]. It has also been demonstrated that HoloTC is a more sensitive marker of vitamin B_{12} status compared with total serum cobalamin, and it could be the earliest and most sensitive marker for vitamin B_{12} deficiency [15].

However, we observed that supplemented subjects had higher levels of plasma tHcy than nonsupplemented patients; this may suggest an involvement of other factors such as genetic, dietary and lifestyle factors as well as pharmacological therapies that may interfere with Hcy metabolism [8].

Besides dietary predictors of plasma tHcy, such as folate, vitamins B_2, B_6 and B_{12}, consistent evidence exists of the interaction with lifestyle factors, including consumption of alcohol and coffee as well as smoking habits, which influences the levels of vitamins metabolically related to Hcy. It has been demonstrated that plasma tHcy levels correlate with the intake of animal source foods, the only natural abundant source of vitamin B_{12} [17], while low intake of fruit and vegetables, natural folate sources, may be associated with inadequate folate intake [12, 25].

As far as our study population is concerned, most of our patients (about 78%) consumed meat and/or fish at least 3 times per week but only about 40% consumed raw leafy vegetables every day indicating that more than half of them were not in line with WHO guidelines [26]. s-F levels were not influenced by *MTHFR* mutation and daily consumption of raw leafy vegetables, while folic acid

supplementation has been shown to be an independent factor for the increase in levels of s-F.

Concerning lifestyle, previous intervention studies showed high levels of coffee consumption associated with higher tHcy levels [27], while quitting regular daily coffee consumption is associated with tHcy decrease [28]. This may be partly due to caffeine and partly to the presence of chlorogenic acid [29] that may also contribute to the effect on levels of tHcy [30]. Alcohol consumption can reduce the level of S-adenosylmethionine (the major methyl donor) and the activity of 5-methyltetrahydrofolate-homocysteine methyltransferase (the enzyme catalyzing the remethylation of Hcy to methionine)| [31]. Habitual smoking is independently associated with hyperhomocysteinemia [32]. Several mechanisms, such as decreased dietary intake, reduced absorption, diminished hepatic uptake, increased urinary excretion as well as a possible interaction between chemical components of cigarette smoke and folate coenzymes, may explain folate deficiency in smokers [33]. In our study, coffee consumption, alcohol and smoking were not independent predictors of increased plasma tHcy; however, the limited sample size and sample of those who consumed alcohol may have influenced statistical significance.

It has been reported that deficiency of female sex hormones is involved in the increase in Hcy levels [22]. Estrogen deficiency in TS, due to X chromosome monosomy, could be a possible cause of hyperhomocysteinemia. Most of our patients were on HRT in contrast to previous findings [22]. Hcy levels were significantly decreased and below the cutoffs, confirming previous findings that report a positive association between Hcy levels and HRT [34].

We recognize that this study has some limitations. First, it is a retrospective study and typically only association and not causation can be inferred from these results. Secondly, the sample size is small but consistent with that of other studies of this specific population; moreover, the data refer to a single center. Finally, evaluations of eating habits and lifestyle were investigated using phone interviews.

This study also has some strengths. In fact, this is the first pilot study to investigate Hcy metabolism, taking into account HoloTC, the transcobalamin-cobalamin complex representing the biologically active form of the vitamin B_{12}, that is an additional and more clinically meaningful parameter than the measurement of total vitamin B_{12} alone [15]. Additionally, to the best of our knowledge, this is the first study to investigate TS patients for eating habits, lifestyle and supplements influencing levels of plasma tHcy.

Conclusion

The well-known cardiovascular risk of TS patients can be reduced using educational approaches to a healthy lifestyle with dietary guidelines that take into account the possible food sources of B vitamins as well as proper supplementation. Good communication is needed in order to involve patients and to inform them about the benefits of adherence. Assessing adherence and discussing any difficulties with it should be part of the continuous health care of these patients. Besides this, we strongly recommend measuring HoloTC which is an early indicator of low B_{12} levels and may be used as a complementary diagnostic strategy for the prompt detection of B_{12} deficiency. Additionally, due to the influence of hormone therapy on Hcy levels, it is mandatory to consider HRT in the tHcy biochemical assessment in TS.

Statement of Ethics

All patients were provided with a consent form at the time of their visit addressing privacy and confidentiality and were invited to sign an agreement authorizing the use of their medical records for research purposes. Ninety-two percent of patients authorized the use of their medical records for this purpose. The study was performed according to the Declaration of Helsinki; Institutional Review Board approval for retrospective data collection was obtained per institutional guidelines.

Disclosure Statement

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