JBUON 2021; 26(3): 1016-1021
ISSN: 1107-0625, online ISSN: 2241-6293 • www.jbuon.com
Email: editorial_office@jbuon.com

ORIGINAL ARTICLE

Parvovirus B19 detection analysis in thyroid tissue paired samples: an observational study from a tertiary surgical oncology department

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Summary

Purpose: *In the present study, we sought to investigate the presence of Parvovirus B19 in both abnormal and normal adjacent thyroid tissue specimens after total thyroidectomy as well as the extent that this phenomenon occurs in a population group referred to a tertiary surgical oncology department.*

Methods: *We detected Parvovirus B19 by Real-Time PCR in both abnormal and normal adjacent thyroid tissue specimens from 41 patients who underwent total thyroidectomy for thyroid disease (cancerous or benign). Hashimoto's thyroiditis, thyroid gland weight, maximum size of the predominant thyroid nodule as well as sex and age of the patients were also evaluated in respect to the Parvovirus B19 presence.*

Results: *Parvovirus B19 virus genome was detected in 21/41 (51.2%) patients in at least one of the paired thyroid tissue samples. No statistically significant difference was noted regarding the sex, age, postoperative diagnosis, thyroid weight and maximum nodule diameter and presence of multifocal disease. The correlation between the incidence of Hashimoto thyroiditis and absence of Parvovirus B19 genome was statistically significant.*

Conclusion: *Our findings showed high prevalence of Parvovirus B19 DNA in thyroid tissue disease in the population examined. Its actual role of the virus and its potential implication in the development or progression of thyroid diseases remain to be elucidated. Larger cohort studies are needed in order to validate a quasi-mutually exclusive role of Hashimoto's thyroiditis and Parvovirus B19 presence in thyroid disease in terms of geographical distribution.*

Key words: *Parvovirus B19, thyroid cancer, Hashimoto's thyroiditis, Real-Time PCR*

Introduction

During the last decades and mainly due to the widespread use of neck ultrasound and its availability and better sensitivity, there has been noted an increase in thyroid nodule detection in the general population. Although death from thyroid cancer is rare, certain carcinogenic risk factors have been proposed such as ionizing radiation at a young age and family history. The most common variation of thyroid cancer is papillary thyroid carcinoma (PTC), accounting for approximately 80% of all cancer cases [1]. Although multinodular goiter (MNG) is classically considered as a benign thyroid disease with an estimated prevalence of approximately 4% in western countries, recent data support the existence of a possible higher cancer risk, close to that of a solitary thyroid nodule [2].

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Received: 07/11/2020; Accepted: 11/12/2020

Parvovirus B19 (also known as Erythrovirus B19) (B19V) is a member of the Parvoviridae family, consisting of a 5.6 kb linear single-stranded DNA genome. B19V is a non-enveloped virus with a diameter of approximately 23–26 nm [3]. B19V infection can cause numerous diseases in humans, such as transient aplastic crisis, persistent anemia in immunocompromised patients, cardiomyopathy and inflammation of various other tissues [4-6]. Two capsid proteins (VP1 and VP2) and one large, nonstructural protein (NS1) are encoded by the B19V genome [7].

The first report that associated B19V infection to PTC was published in 2008 [8]. The authors reported the presence of Parvovirus B19 sequences in PTC paraffin-embedded thyroid samples as well as the presence of capsid protein. Additional data were available in 2011 when Parvovirus B19 infection was reported in both anaplastic thyroid carcinoma (ATC) and Hashimoto's thyroiditis (HT) in archived thyroid tissue samples [9]. The same group in 2014 reported for the first time, the presence of B19V DNA, mRNA and capsid proteins not only in thyroid cancer cells but also in normal thyroid tissue cells with no statistically significant difference [10]. Moreover, in a recent study, B19V detection through immunohistochemistry (IHC) was found not to be statistically significant among Grave's disease thyroid tissue specimens and multinodular goiter thyroid tissue specimens [11].

Although an emerging number of studies during the last decade imply a possible role of B19V presence in thyroid disease, there is conflicting evidence considering the presence of B19V in thyroid cancer cells and normal thyroid epithelial cells. We sought to investigate the presence of Parvovirus B19 in thyroid tissue specimens after total thyroidectomy for both a cancerous or a benign diagnosis and the extent that this phenomenon occurs in the population group referred to a tertiary surgical oncology department.

Methods

Subjects and design of the study

The study was approved by the Ethics Committee of Saint Savvas Cancer Hospital, Athens, Greece.

In the present study, 41 patients who underwent total thyroidectomy at the surgical oncology department of the "Saint Savvas Cancer Hospital", Athens, Greece, agreed to participate and were enrolled over a 3-year period (2016-2019). Thyroid tissue samples were obtained intra-operatively after total thyroidectomy and thyroid gland weight measurement was assessed. After close inspection, a sample of the abnormal thyroid tissue and the adjacent normal thyroid tissue was obtained and stored at -80°C, creating a pool of total 82 thyroid tissues samples. All abnormal tissue samples were re-examined by pathologists to confirm the diagnosis. Enrollment criteria were: suggestive fine needle aspiration (FNA) cytology and an ultrasonography mapping of the thyroid gland. Exclusion criteria were: immunosuppression and glucocorticoid treatment during the last three months before surgery. All patients had good hormone functionality at the time of surgery.

Thirteen males (31.7%) and 28 (68.3%) females with a mean age of 52.6 ± 12.5 years were fulfilling the inclusion criteria of the study. In respect of sex differences, the mean age of the males was 50.7 ± 12.4 years whereas the mean age of the females was 53.5 ± 12.7 years.

Twenty-six out of 41 patients (63.4%) were admitted to the operating room with a preoperative diagnosis of multinodular goiter and 11 patients (26.9%) were admitted to the operating room with a preoperative diagnosis of papillary thyroid cancer after a suggestive fine needle aspiration of the thyroid gland. Four patients (9.7%) presented with cellular atypia in the preoperative FNA cytology report.

The study was approved by the Scientific Board of the "Saint Savvas Cancer Hospital", Athens, Greece and informed consent was obtained from all the patients that were assigned in this study. Helsinki declaration amendments and personal data safety are research priorities for all study phases, before and after data publication.

Nucleic acid isolation

Thyroid tissue samples were homogenized and DNA extraction was carried out using the Genomic DNA Purification Kit (Genomed, Löhne, Germany) according to the manufacturer's instructions. DNA was rehydrated with hydration buffer and stored at -20°C until use. RNA extraction was carried out using the Trizol (Invitrogen, Grand Island, NY) protocol. Nucleic acid quality was confirmed by its absorption rate at 260/280nm using a ND1000D spectrophotometer (NanoDrop, Wilmington, DE).

Real-time polymerase chain reaction

Determination of the B19 presence was achieved by Real-Time PCR using the Parvovirus B19 Real-TM Quant kit (Sacace Biotechnologies Srl, Italy) according to the manufacturer's protocol [12]. Specifically, we used the following thermal cycling profil: 1 cycle at 95°C for 15 min; 5 cycles at 95°C for 5 s, at 60°C for 20 s, at 7 °C for 15 s; and 40 cycles at 95°C for 5 s, at 60°C for 30 s and 72 °C for 15 s. The internal control (IC) and negative control (NC) buffer included in the kit were used to validate the qualitative and quantitative analysis respectively.

Statistics

All categorical variables are summarized as number of counts and percentage. All numerical variables are being presented as median (min, max, Inter-quartile range [IQR]. Univariate comparisons between categorical variables were conducted using Pearson's chi-square test and Fisher's exact test (Table 1). For comparisons between categories with many empty cells the p value

for the linear-by-linear association is reported instead of the Chi-square (Table 2). Univariate comparisons in cases of numeric variables were performed using the Mann-Whitney or Kruskal-Wallis tests. The level of significance was set to α=0.05 and the IBM SPSS Version 24.0 (Statistical Package for social Sciences for Windows 24 Inc., Chicago, II, USA) was used for the analysis.

Results

We investigated the B19V presence in both the abnormal thyroid tissue and normal adjacent thyroid tissue in 41 patients. In Table 1, the demographic data in respect to B19V presence are presented.

B19V was detected in thyroid tissue samples in 21 (51.2%) patients in at least one of the paired thyroid tissue samples. We furthermore subdivided the B19V presence group into three subgroups regarding the specific presence of B19V in either the abnormal thyroid tissue alone, the normal adjacent thyroid tissue alone and in both the abnormal and normal adjacent thyroid tissue, as shown in Table 2. In 2 out of 21 (9.5%) patients, B19V was present in the abnormal thyroid tissue alone whereas in eleven out of 21 (52.4%) patients B19V was present in the normal adjacent thyroid tissue alone. In the remaining 8 out 21 (38.1%) patients, B19V was detected in both the abnormal thyroid tissue and the normal adjacent thyroid tissue.

As a next step, we sought to investigate the correlation between B19V and the sex of the patients. Parvo B18 was not detected in 15 out of 28 (53.5%) females and in 5 out of 13 (38.4%) males. No statistically significant difference was noted regarding the sex of the patients (p=0.811).

As regards the age, in the B19V absence group, the median age was 57.5 (range:21-79) years old and in the B19V presence group the median age was 53.0 years old (range:27-68). Although there was noted a positive trend towards the likelihood of B19V presence as age increases, the difference was not statistically significant despite a tendency towards significance (p=0.070).

Regarding the postoperative diagnosis and after obtaining the definite histopathology report, we categorized the patients into three groups: a) of multinodular goiter (MNG) with a total of 19 out of 41 (46.4%) patients, b) papillary thyroid cancer (PTC) with a total of 22 out of 41 (51.2%) patients and c) a benign thyroid nodule in 1 (2.4%) patient. Our statistical analysis retrieved no significant correlation among the patients groups in respect to the definite postoperative diagnosis.

We next investigated the thyroid's gland total weight in grams. The median thyroid weight for the B19V presence group was 22 gr (range:10-210) and for the B19V absence group was 33.5 gr (range:10-114). The statistical analysis showed that the difference was not statistically significant (p=0.175).

Table 1. Patients and thyroid tissue demographic data in respect to B19V presence

Demographic data	*B19V absence (n=20)*	*B19V presence (n=21)*	*p value*
Gender			0.368
Females, n (%)	15 (75.0)	13 (61.9)	
Males,n (%)	5 (25.0)	8 (38.1)	
Age (years)			0.070
Median (min, max; IQR)	57.5 (21, 79; 14)	53.0 (27, 66; 18)	
Post-op diagnosis			0.548
MNG, n (%)	9 (45.0)	10 (47.6)	
PTC, n (%)	10 (50.0)	1152,4)	
Benign nodule, n (%)	1 (5.0)	0 (0.0)	
Weight (gr)			0.175
Median (min, max; IQR)	33.5 (10, 114; 26)	22 (10, 210; 15)	
Hashimoto's thyroiditis			0.045*
Yes, n (%)	6 (30.0)	1 (4.8)	
Max diameter (mm)			0.320
Median (min, max; IQR)	18.5 (6, 50; 16)	12 (3, 40; 11)	
Multifocal disease			0.719
Yes, n (%)	5 (25.0)	4 (19.0)	

*Fisher's exact test

Table 2. Patients and thyroid tissue demographic data in respect to B19V presence among specific subgroups

	B19V absent	*B19V present*			*p value*
	in both abnormal and normal tissue (n=20)	*in abnormal tissue only (n=2)*	*in normal tissue only n=11)*	*in both abnormal and normal tissue (n=8)*	
Sex					0.811
Females, n (%)	15 (75.0)	1 (50.0)	7 (63.6)	5 (62.5)	
Males, n (%)	5 (25.0)	1 (50.0)	4 (36.4)	3 (37.5)	
Age (years)					0.279
Median (min, max; IQR)	57.5 (21, 79; 14)	54.0 (48, 60; 12)	53.0 (37, 66; 20)	51.0 (27, 61; 21)	
Post-op diagnosis					0.930
MNG, n (%)	9 (45.0)	1 (50.0)	6 (54.5)	3 (37.5)	
PTC, n (%)	10 (50.0)	1 (50.0)	5 (45.5)	5 (62.5)	
Benign nodule, n (%)	1 (5.0)	0 (0.0)	0 (0.0)	0 (0.0)	
Weight (gr)					0.350
Median (min, max; IQR)	33.5 (10, 114; 26)	34.0 (22, 46; 24)	22.0 (10, 33; 15)	39.8 (12, 220; 20)	
Hashimoto's thyroiditis					0.039*
Yes, n (%)	6 (30.0)	0 (0.0)	1 (9.1)	0 (0.0)	
Max diameter (mm)					
Median (min, max; IQR)	18.5 (6, 50; 16)	18.5 (15, 22; 7)	12.0 (3, 30; 14)	14 (8, 40; 11)	0.656
Multi-focal disease					0.736
Yes, n (%)	5 (25.0)	0 (0.0)	2 (27.3)	1 (12.5)	

*linear-by-linear association

Our analysis was further expanded in investigating the maximum diameter of the predominant thyroid nodule and its correlation with the B19V status. The median value of the maximum thyroid nodule diameter for the B19V presence group was 12 mm and for the B19V absence group was 18.5 mm. Our analysis showed that the difference was not statistically significant (p=0.32).

Regarding the multifocality of the disease in the PTC thyroid specimens, our statistical analysis has showed that the multifocality of the disease was noted in a total of 5 (25.0%) patients in the B19V absence group and in 4 (19.0%) patients in the B19V presence group, however, the difference was not statistically significant.

Finally, we studied the role of Hashimoto's thyroiditis and its correlation with B19V presence in our thyroid tissue samples. Hashimoto's thyroiditis was diagnosed in a total of 6 (30.0%) patients in the group of patients with no detection of B19V and in only one (4.8%) patient in the B19V presence group. Interestingly, our statistical analysis showed that there is a statistically significant difference in Hashimoto's thyroiditis presence and B19V absence (p=0.045).

Discussion

In the present study, we examined the Parvovirus B19 presence in postoperative thyroid tissue specimens of 41 patients who underwent total thyroidectomy under a cancerous or a benign preoperative diagnosis, in a tertiary surgical oncology department. We tested both the abnormal thyroid tissue and the adjacent normal thyroid tissue of each patient. Within half of the samples examined detection of B19V has been reported.

Although Parvovirus B19 is considered to be an apoptosis inducing virus (13), recent studies have shown a possible effect in thyroid disease and particularly in thyroid cancer [8,14]. Parvovirus B19 sequences were first reported in 2008 by Wang et al in PTC paraffin-embedded thyroid samples using immunohistochemistry (IHC), nested PCR and *in situ* hybridization (ISH) methods in 63%, 83.3% and up to 97% of cases. In 2011, Parvovirus B19 presence was reported by Adamson et al in both anaplastic thyroid carcinoma (ATC) and Hashimoto's thyroiditis (HT) in archived thyroid tissue samples [9]. The same authors showed in 2014, the presence of B19V DNA, mRNA and capsid proteins not only

in thyroid cancer cells but also in normal thyroid tissue cells with no statistically significant difference [10]. Parvovirus B19 is an obligatory human pathogen and studies have shown that it persists in other than thyroid, human tissue such as the heart and the testis. This relatively frequent presence of B19V in these tissues was reported not to be a disease related [16-18]. Adamson et al [10] showed the presence in normal thyroid tissue and that normal thyroid epithelial cells express the receptors needed for the B19V. This is an important first step towards the theory of Mori et al [19] for the innocent bystander B19V in thyroid tissue and that its presence is not affecting or triggering pathologic mechanisms in thyroid gland. Indeed in our prospectively designed study it is shown that B19V is present not only in abnormal thyroid tissue samples but also in the adjusting normal thyroid tissue in thyroid glands, after total thyroidectomy for a cancerous or a benign disease state. The host cell for Parvovirus B19 is the bone marrow erythroid progenitor cell although it may persist in various other human tissues. In a recent study, the authors argue that the detection of B19V DNA in blood by PCR could refer to DNA strands released from tissues without active replication [19]. The authors state that the B19V presence, although it has been confirmed in non-permissive for B19V cells, these tissues have been reported to contain B19V DNA and the persistence could be episomal, due to the lack of certain mechanisms to degrade this viral DNA. Our subgroup analysis revealed that B19V was present in both the abnormal and the normal adjacent thyroid tissue in 8 out of 21 (38.1%) patients. Moreover, in 11/21 (52.4%) patients B19V was detected in only the normal adjacent thyroid tissue but not in the abnormal thyroid tissue. Finally, in 2/21 (9.5%) patients, B19V was detected in the abnormal thyroid tissue but not in the adjacent normal thyroid tissue. It is difficult to yet describe which hypotheses could explain viral persistence within normal and abnormal tissues.

It has been reported that nodule incidence increases as age increases and particularly in women and the nodule size is inversely correlated to the malignancy risk [21-23]. In our study although there was noted a positive trend towards the likelihood of B19V presence as age increases, a greater size of sample collection may be needed.

Although various reports during the last two decades have shown B19V presence in thyroid specimens with thyroiditis, a clear link is yet to be established (14,19,24,25). In a study of Mori et al [26] the authors introduced the non-structural protein 1 (NS1) gene of B19 into C57BL/6 mice and provoked thyroiditis via thyroglobulin (Tg) immunization. Thyroiditis was induced in a quarter of the NS1-transgenic subjects, but failed to be induced in wild-type subjects and this difference was did not statistically significant. In our present study we examined Hashimoto's thyroiditis presence and its correlation with the B19V presence. B19V was present in only one patient (4.8%) with Hashimoto's thyroiditis whereas B19V was not detected in 6 (30%) patients with Hashimoto's thyroiditis. Extremely careful assumptions should be made because of the small number of enrolled patients, especially when environmental or socio-demographic determinants may lead to further sub-grouping of not easily collected surgical samples [27]. Geo-spatial observations based on numerically more robust research initiatives are needed to confirm our hypothesis.

It is obviously a demanding task to link a virus which is an obligatory human pathogen with cancer, since animal models and *in vitro* analysis in cell lines can only raise the suspicion. However, its presence within normal tissue at an 'innocent' time point can be easily shown. A hypothesis that could explain discrepancies across all models of investigation may possess more plausibility. Additional studies in human thyroid tissue specimens are needed to elucidate the role of B19V in thyroid disease.

Authors' contributions

DN performed the experiments and wrote the manuscript. AZ designed and performed experiments while he also contributed to the manuscript. EKS and AB statistically analysed the results and contributed to the manuscript, SD performed experiments, DAS conceived experiments and edited the manuscript, GS surgically obtained the biological material and GS conceived and designed experiments as well as he edited the manuscript. All authors read and approved the final manuscript.

Conflict of interests

The authors declare no conflict of interests.

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