Correlation between serum 25-(OH)D_3 level and immune imbalance of Th1/Th2 cytokines in patients with Hashimoto's thyroiditis and its effect on autophagy of human Hashimoto thyroid cells

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Received July 16, 2020; Accepted December 18, 2020

DOI: 10.3892/etm.2021.9889

Abstract. The study aimed to determine the relationship between serum 25-(OH)D_3 and Th1/Th2 cytokine immune imbalance, and the effect of 25-(OH)D_3 on the autophagy of human Hashimoto thyroid cells. Western blot analysis was used to detect the expression levels of microtubule-associated protein 1 light chain 3 (LC3) and autophagy-associated protein mammalian target protein of rapamycin (mTOR) in thyroid tissues of 20 Hashimoto's thyroiditis (HT) patients and normal tissues of 20 benign thyroid adenomas. Nthy-ori3-1 cells (normal cells of human thyroid follicular epithelium) were treated with different concentrations of 25-(OH)D_3 for 24 h. The expression of LC3, mTOR and caspase-3 protein in the cells was detected by western blot analysis. The apoptosis and proliferation levels were detected by flow cytometry and MTT assay, respectively. The levels of FT3, FT4 and IL-10 in the HT group were lower than those in the healthy control group. The serum levels of 25-(OH)D_3, TPOAb and TGAb in the HT group were lower than those in the healthy control group. Serum 25-(OH)D_3 level in the HT group was negatively correlated with IL-2 and IFN-γ, and positively correlated with IL-4. In Hashimoto's thyroiditis tissues, the expression of mTOR was higher while the expression of LC3B-II was lower than that of normal thyroid tissue. With the increase in 25-(OH)D_3 concentration, the expression level of mTOR increased, the expression level of LC3B-II decreased and the apoptosis rate was significantly increased. The cell proliferation rate decreased with the increase in 25-(OH)D_3 concentration. The serum 25-(OH)D_3 level in HT hypothyroidism patients was significantly lower than that of the control group. Thus, 25-(OH)D_3 may be involved in the disease progression by upregulating the levels of Th1 cytokines and downregulating the levels of Th2 cytokines. 25-(OH)D_3 can inhibit autophagy of thyroid cells, induce apoptosis and participate in the pathogenesis of Hashimoto's thyroiditis.

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Key words: Hashimoto's thyroiditis, Th1/Th2 cytokines, 25-(OH)D_3, autophagy, proliferation, apoptosis

Introduction

Hashimoto's thyroiditis (HT) is characterized by high prevalence, hidden onset and slow progress. At present, the relationship between vitamin D and HT has become a hot spot at home and abroad, but the mechanism has not yet been clarified. Studies have shown that there exist the upregulation of Th1 cytokines, downregulation of Th2 cytokines, transformation and interaction between TH1 and Th2 in HT (1,2). Therefore, intervention in the differentiation and selection of Th cell subsets may be helpful to the treatment of autoimmune reaction. Vitamin D and its receptor-mediated biological effects are involved in many pathophysiological processes, such as regulation of calcium and phosphorus metabolism, immune regulation, anti-inflammation, anti-infection, and tumor prevention. 25-Hydroxyvitamin D_3 [25-(OH)D_3] is an active form of vitamin D. Studies have found that 25-(OH)D_3 inhibits Th1 cell activity and regulates Th1/Th2 cell imbalance (3,4) and is beneficial to the maintenance of intact thyroid tissue morphology in rats (5,6). Correcting the imbalance of Th1/Th2 and adjusting the immune response in HT patients has important clinical research value (7,8). Autophagy is a normal metabolic pathway to maintain the amount of eukaryotic cells, which is mainly mediated by lysosomes. Its dysfunction is related to tumor and infection. Studies have shown that vitamin D/vitamin D receptor (VDR) can influence the progression of autoimmune thyroid diseases by regulating autophagy (9-14). In the present study, the immune mechanism related to vitamin D and HT was investigated. Human thyroid cells were selected to study the effect of 25-(OH)D_3

on autophagy, apoptosis and proliferation of cells, which may provide a new treatment strategy for clinical HT.

Materials and methods

Research participants. Fifty newly diagnosed HT patients (age range, 30-60 years) at The Second Affiliated Hospital Of Qiqihar Medical University (Qiqihar, Heilongjiang, China) from February 2019 to December 2019 were selected as the HT group. There were 15 males and 35 females, with a mean age of 38.91±6.53 years.

The inclusion criteria of the HT group were as follows: i) Patients with diffuse or local enlargement of thyroid gland; ii) anti-thyroglobulin antibodies (TGAb) and thyroid peroxidase antibody (TPOAb) were both positive; iii) patients accompanied by hypothyroidism, serum TSH level was higher than normal. Exclusion criteria were as follows: i) Pregnant, lactating and menopausal women; ii) dysfunctional or exhausted heart, lung, brain, liver, kidney or other important organs; iii) patients suffering from other autoimmune endocrine diseases; iv) patients with thyroid cancer; v) patients who previously used immunoenhancers or immunosuppressants within three months; vi) patients with a history of vitamin D replacement therapy in the past 12 months.

Fifty healthy individuals (30-60 years) who met the physical health standards at our hospital from February 2019 to December 2019 were selected as the normal control (NC) group. There were 17 males and 33 females, with a mean age of 39.57±10.04 years. Exclusion criteria were as follows: Patients with abnormal thyroid function; pregnant women, lactating women, menopausal women or women with other endocrine diseases, autoimmune diseases or systemic diseases (tumor, asthma or other allergic diseases, active infections); patients with other endocrine diseases and autoimmune diseases; patients who took drugs in the previous three months and had a history of vitamin D replacement therapy in the past 12 months.

All subjects signed informed consent. The present study was approved by the Ethics Committee of The Second Affiliated Hospital Of Qiqihar Medical University (Qiqihar, Heilongjiang, China) after examination.

Materials. Following informed consent of the patients, another 20 cases of thyroid adenoma with Hashimoto's thyroiditis were collected as the case group, and 20 cases of normal tissues adjacent to benign thyroid adenoma were collected as the control group. Nthy-ori3-1 cells (normal cells of human thyroid follicular epithelium) were purchased from the Wuhan Cell Bank of the Chinese Academy of Sciences. The rabbit anti-β-actin antibody (ab179467), rabbit anti-LC-3 antibody (ab48394), rabbit anti-mTOR antibody (ab109268) and rabbit anti-caspase-3 antibody (ab197202) were all purchased from Abcam. RPMI-1640 culture medium was purchased from BD Biosciences. Fetal bovine serum (FBS) was purchased from Gibco (Thermo Fisher Scientific, Inc.). The MTT cell proliferation kit (cat. no. 4890-025-k) was purchased from Trevigen, USA. 25-(OH)D_3 was purchased from Shanghai Yubo Biotechnology Co., Ltd. (D455087). Double-distilled water (ddH_2O) was used to prepare 20, 40, 60, 80 and 100 mmol/l solutions.

Methods

Determination of relevant indexes. Levels of five free thyroid functions: Free triiodothyronine (FT3), free thyroxine (FT4), anti-thyroglobulin antibodies (TGAb), thyroid peroxidase antibodies (TPOAb), and the third generation thyroid-stimulating hormone (s-TSH) were detected by a full-time examiner through chemiluminescence immunoassay analyzer (IMMULITE 2000; Siemens, Germany).

Determination of 25-(OH)D_3. We collected venous blood from the patients on an empty stomach in the morning, which was placed in a serum separation glue test tube, and let stand at room temperature for 1 h. After centrifugation, we took the upper serum, and added it to the 25(OH)D_3 kit (ZCi Bio, cat. no. ZC-31637). We applied the double antibody sandwich enzyme-linked immunosorbent (ELISA) for detection, using a microplate reader (Shanghai Kehua Bio-engineering Co., Ltd.). All procedures were strictly in accordance with the specifications included in the kits.

Determination of the cytokines. The related cytokine kits purchased from Beijing Keruimei Technology Co., Ltd. (Hu IL-2, 850.010.X; Hu IL-4, 950.020.X; Hu IL-6 CE, 950.030.X; Hu hs IL-10 kit, 850.880.X; Hu IFN-γ kit CE, 950.000.X) and serum 25-(OH)D_3 detection kit (Shanghai Beyotime Biotechnology Co., Ltd., ZC-31637) were used according to the manufacturers' instructions.

Cell culture. Nthy-ori3-1 cells (human thyroid follicular epithelial normal cells) were purchased from Wuhan Cell Bank of the Chinese Academy of Sciences. The cells were placed in RPMI-1640 culture medium containing 10% FBS at 37˚C with 5% CO_2. When the cells grew to a logarithmic growth stage, they were inoculated into a 6-well plate. Then the culture continued for 24 h with different concentrations of 25-(OH)D_3 (0, 20, 40, 60, 80 and 100 mmol/l) for subsequent experimentation.

Effect of 25-(OH)D_3 on the expression of mTOR and LC3B-II in cells by western blot analysis. Cells were digested with trypsin, washed with PBS, centrifuged at 12,000 x g at 37˚C for 10 min, fully lysed with lysis solution, and centrifuged to obtain the supernatant. The protein concentration was determined by BCA assay. SDS-PAGE protein loading buffer (5X) was prepared, and the protein was denatured by boiling water bath for 10 min. Cooling, loading, and electrophoresis were performed respectively. Proteins (10 μl) were transferred to PVDF membranes, with the current of 15-20 mA overnight. Commassie blue staining solution was used for rapid staining. PAGE membrane was rinsed, blocking solution was added and incubated overnight on a shaking table at room temperature. Then the blocking solution was removed, and the primary antibody (1:500) was added and incubated overnight at 4˚C on a shaking table. The sample was then washed, goat anti-rabbit secondary antibody (HRP labeled, 1:6,000), was added and incubation was carried out at 37˚C for 1 h. The secondary antibody was recovered and washed 3 times. BeyoECL Plus (Beyotime Biotechnology, Inc.) was used to detect protein; pressing plate and rinsing were performed. Protein expression was represented by absorbance of target protein/absorbance of internal reference (GADPH), using ImageJ v1.8.0 software (National Institutes of Health, Bethesda).

Table I. Comparison of the general data and five free thyroid functions between the HT and NC group.

Items	HT group (n=50)	NC group (n=50)	t/χ^2	P-value
Age (years)	38.91±6.53	39.57±10.04	0.399	0.691
Sex (M/F)	15/35	16/34	0.047	0.829
BMI (kg/m^2)	29.17±4.89	28.93±3.09	0.293	0.770
s-TSH (mIU/l)	10.09±3.81	2.31±1.32	13.643	<0.01[a]
FT3 (pmol/l)	5.09±1.02	6.38±1.29	5.547	<0.01[a]
FT4 (pmol/l)	12.09±7.02	17.02±1.07	4.909	<0.01[a]
TPOAb (IU/ml)	801.01±400.23	9.02±3.18	13.975	<0.01[a]
TGAb (IU/ml)	203.71±109.32	16.09±2.46	12.164	<0.01[a]

[a]P<0.01, HT vs. the NC group. HT, Hashimoto's thyroiditis; NC, normal control; BMI, body mass index; FT3, triiodothyronine; FT4, free thyroxine; TGAb, anti-thyroglobulin antibodies; TPOAb, thyroid peroxidase antibodies; s-TSH, thyroid-stimulating hormone.

Table II. Comparison of serum 25-$(OH)D_3$ and T-helper cytokines between the HT and NC group.

Indicators	HT group (n=50)	NC group (n=50)	t	P-value
25-$(OH)D_3$ (ng/ml)	19.52±3.89[b]	16.28±3.94	3.793	<0.01[b]
IL-2 (pg/ml)	4.09±1.24[a]	2.18±1.13	8.050	<0.01[b]
IL-4 (pg/ml)	2.13±1.20	2.29±1.32	0.634	0.528
IL-6 (pg/ml)	1.65±0.19	1.59±0.32	1.140	0.257
IL-10 (pg/ml)	1.99±0.23[b]	2.98±0.52	12.311	<0.01[b]
IFN-γ (pg/ml)	12.87±1.63[b]	3.89±2.01	24.536	<0.01[b]

[a]P<0.05, [b]P<0.01, HT vs. the NC group. IL, interleukin; IFN interferon.

Detection of apoptosis as detected by Annexin V-PE/7-AAD double staining method. 25-$(OH)D_3$ was added to treat the cells for 24 h, and then the cells were collected. Binding buffer (100 µl) was then added to each well, and 5 µl Annexin V-PE was added, and incubation was carried out at 4˚C in the dark for 15 min. 7-AAD (10 µl) was added and incubation was carried out for 5 min in the dark, and 400 µl binding buffer was added. Then flow cytometry was used, in which Annexin V-PE was detected through FL1 channel and 7-AAD was detected through FL3 channel.

Cell proliferation as determined by MTT assay. The cells in the logarithmic growth phase were added to 96-well plates, at $5x10^3$ cells per well and 4 multiple wells in each group. The cells were cultured in an incubator for 2 h. 25-$(OH)D_3$ at different concentrations was added to each well and cultured for 24 h. The 96-well plate was taken out, 20 µl MTT (5 mg/ml) was added to each experimental well and incubated at room temperature for 4 h. The supernatant was removed from each well, and 150 µl DMSO was added to each well. The absorbance (A) of each well at 490 nm was measured, and the cell growth inhibition rate was calculated as: (1-A value of the case group/A value of the control group) x100%.

Statistical analysis. SPSS 20.0 (IBM Corp.) was used for data analysis. The data are expressed as mean ± standard deviation (SD), and the comparison between the two groups was conducted by normality test and variance homogeneity test. The t-test was used when the criteria were met. Analysis of variance (ANOVA) was used for multi-group comparisons. Pearson analysis and multiple stepwise regression analysis were used to analyze the correlation of each molecule. GraphPad Prism 5.0 (GraphPad Software, Inc.) was used for figure analysis. A difference was deemed statistically significant at P<0.05.

Results

Comparison of general data and five free thyroid functions between the HT and NC group. As documented in Table I, statistical analysis showed that there was no statistical difference in age, sex and body mass index (BMI) between the two groups (P>0.05). The level of s-TSH in the case group was significantly higher than that in the control group (P<0.01). The levels of FT3 and FT4 in the HT group were significantly lower than those in the NC group. Rank sum test analysis showed that there was a significant difference between the two groups (P<0.01). The levels of TPOAb and TGAb in the HT group were significantly higher than those in the NC group, and rank sum test analysis showed that there were significant differences between the two groups (P<0.01).

Comparison of serum 25-$(OH)D_3$ and Th1 and Th2 cytokines between the HT and NC group. As documented in Table II,

Table III. Correlation of indexes in the HT group.

Item	25-(OH)D_3	s-TSH	TPOAb	TGAb	FT4	FT3
IL-2	r=-0.602	r=-0.481	r=0.385	NS	r=-0.495	r=0.368
	P<0.001[b]	P=0.006[a]	P=0.031[a]		P=0.004[b]	P=0.039[a]
IL-4	r=0.507	NS	NS	NS	NS	NS
	P=0.002[b]					
IL-6	NS	NS	NS	NS	NS	NS
IL-10	NS	NS	NS	NS	NS	NS
IFN-γ	r=0.605	r=0.627	r=0.663	r=0.396	r=0.533	r=0.516
	P<0.001[b]	P<0.001[b]	P<0.001[b]	P=0.024[a]	P<0.001[b]	P=0.002[a]
25-(OH)D_3	1	r=-0.517	r=-0.701	r=-0.515	r=0.665	r=-0.407
		P=0.002[a]	P<0.001[b]	P=0.003[a]	P<0.001[b]	P=0.022[a]

[a]P<0.05, [b]P<0.01. NS, not significant (P>0.05). HT, Hashimoto's thyroiditis; T3, triiodothyronine; FT4, free thyroxine; TGAb, anti-thyroglobulin antibodies; TPOAb, thyroid peroxidase antibodies; s-TSH, thyroid-stimulating hormone; IL, interleukin; IFN, interferon.

serum 25-(OH)D_3 level in the HT group (19.52±3.89 ng/ml) was higher than that in the control group (16.28±3.94 ng/ml) (P<0.01). Serum interferon (IFN)-γ and interleukin (IL)-2 levels in the HT group were higher than those in the NC group (P<0.01), while serum IL-10 levels in the HT group were lower than those in the NC group (P<0.01). There was no significant difference in serum IL-4 and IL-6 levels between the two groups (P>0.05). This suggested that the serum 25-(OH)D_3 level in patients with HT may be involved in disease progression by upregulating IFN-γ and IL-2 levels and downregulating IL-10 levels.

Correlation analysis in the HT group. As documented in Table III, the level of 25-(OH)D_3 in the HT group was negatively correlated with IL-2 (r=-0.602) and IFN-γ (r=-0.605), and positively correlated with IL-4 (r=0.507). It was negatively correlated with FT3 (r=-0.407), s-TSH (r=-0.517), TPOAb (r=-0.701) and TGAb (r=-0.515), and it had no significant correlation with IL-6 and IL-10. 25-(OH)D_3 was positively correlated with FT4 levels (r=-0.515). s-TSH was positively correlated with IFN-γ (r=0.627) and IL-2 (r=0.481), but not correlated with IL-4, IL-6 and IL-10. TPOAb was negatively correlated with 25-(OH)D_3 (r=-0.701), positively correlated with IFN-γ (r=0.663) and IL-2 (r=0.385), but not significantly correlated with IL-4, IL-6 and IL-10. TGAb was negatively correlated with 25-(OH)D_3 (r=-0.515), positively correlated with IFN-γ (r=0.396), but not significantly correlated with IL-2, IL-4, IL-6 and IL-10.

Multiple stepwise regression analysis. s-TSH in the case group was taken as dependent variable. IFN-γ and IL-2 in the case group were taken as independent variables. The results showed that s-TSH was significantly affected by IFN-γ (P<0.01), while IL-2 did not enter the regression equation.

TPOAb in the HT group was taken as a dependent variable. IFN-γ and IL-2 in the case group were taken as independent variables. The result showed that TPOAb was significantly affected by IFN-γ (P<0.01), while IL-2 did not enter the regression equation.

Expression level of autophagy-related proteins in HT tissues. Western blotting showed that the expression level of mTOR protein in the HT tissues was significantly higher than that in the normal thyroid tissues (Fig. 1A and B) (P<0.05), while LC3B-II protein in the HT tissues was lower than that in normal thyroid tissues (Fig. 1A and C, P<0.05). This suggests that the level of autophagy-related proteins in HT tissues is lower than that in normal tissues.

Effects of different concentrations of 25-(OH)D_3 on the autophagy of thyroid cells. Nthy-ori3-1 cells were treated with 25-(OH)D_3 at different concentrations for 24 h. The expression levels of autophagy proteins, mTOR and LC3B, in each group were detected by western blot analysis. The results of WB experiment showed that with the increase in 25-(OH)D_3 concentration, the expression level of mTOR protein was significantly increased (Fig. 2A and B) and the expression level of LC3B-II protein was significantly decreased, especially when the concentration of 25-(OH)D3 was 60 mmol/l (Fig. 2A and C).

Effects of different concentrations of 25-(OH)D_3 on apoptosis of Nthy-ori 3-1 cells. Nthy-ori 3-1 cells were treated with different concentrations of 25-(OH)D_3 (0, 20, 60, 100 mmol/l) for 24 h, and then the cells were collected to detect the apoptosis rate. The results showed that the apoptosis rate was gradually but significantly increased with the increase in 25-(OH)D_3 concentration (P<0.05), especially when treated with 60 mmol/l (Fig. 3).

Effects of different concentrations of 25-(OH)D_3 on the proliferation of Nthy-ori 3-1 cells. With the increase in the concentration of 25-(OH)D_3, the cell proliferation ability was significantly decreased (increased rate of inhibition), especially when treated with 60 mmol/l. When treated with 80 and 100 mmol/l, the proliferation capacity was not significantly decreased as the inhibition rate decreased. We speculated that excessive 25-(OH)D_3 may produce cytotoxicity when increased to excessively high concentration (Fig. 4).

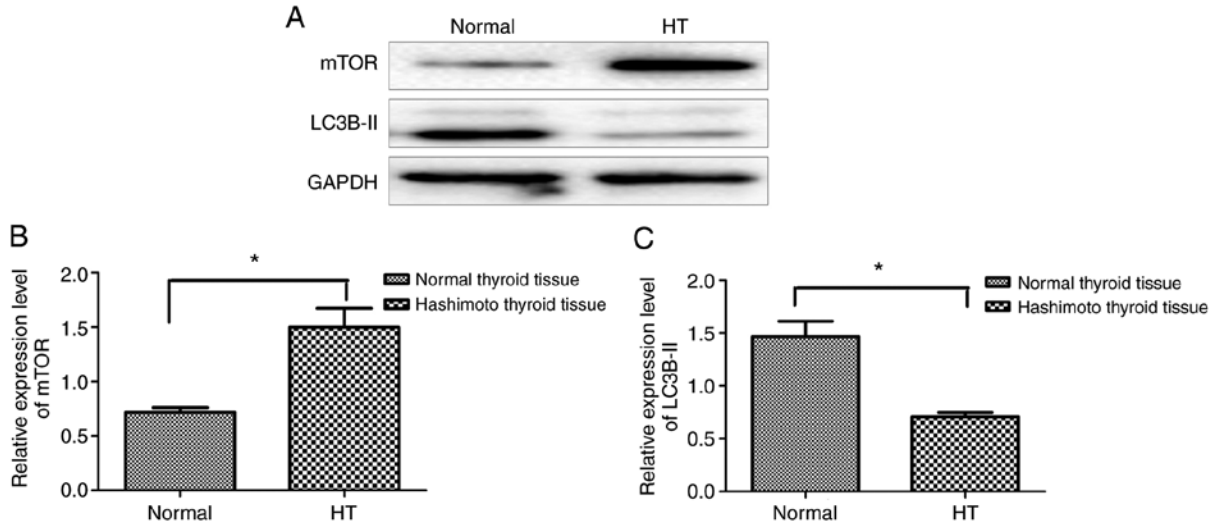

Figure 1. Expression level of autophagy-related proteins in HT. (A) The protein expression of mTOR and LC3B-II in HT and normal tissues by western blot analysis. (B) mTOR expression in HT and normal tissues. (C) LC3B-II expression in HT and normal tissues. *P<0.05. HT, Hashimoto's thyroiditis; LC3-II, microtubule-associated protein 1 light chain 3-II; mTOR, mammalian target protein of rapamycin.

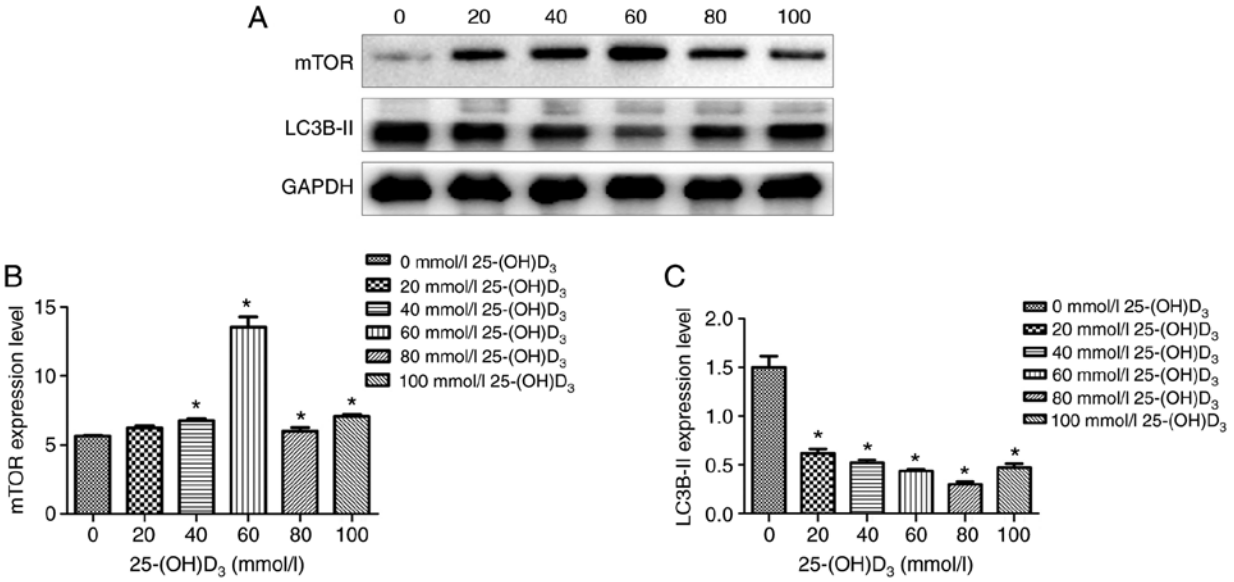

Figure 2. Expression changes of mTOR and LC3B-II in Nthy-ori3-1 cells after treatment with different concentrations of 25-(OH)D_3. (A) Western blot detection of protein expression of mTOR and LC3B-II after treatment of Nthy-ori3-1 cells with different concentrations of 25-(OH)D_3. (B) mTOR expression after treatment of Nthy-ori3-1 cells with different concentrations of 25-(OH)D_3. (C) LC3B-II expression after treatment of Nthy-ori3-1 cells with different concentrations of 25-(OH)D_3. *P<0.05, compared with the untreated control. LC3-II, microtubule-associated protein 1 light chain 3-II; mTOR, mammalian target protein of rapamycin.

Discussion

Hashimoto's thyroiditis (HT) is a common autoimmune thyroiditis (AIT). It has been reported that a variety of cytokines are involved in the occurrence of the disease by alteration of certain functions of immune-competent cells and thyroid follicular epithelial cells. Studies have shown that Th1 and Th2 cytokines play important roles in the occurrence and development of HT (15-17). It was found previously that serum IFN-γ is positively correlated with TGAb and TPOAb, and IL-2 is positively correlated with TGAb and TMAb (18). IFN-γ was found to be highly expressed and IL-4 was lowly expressed in the serum of an autoimmune thyroiditis model (19). The positive expression of IFN-γ in HT patients is significantly higher than that of IL-4, indicating that the thyroid tissue of HT patients is dominated by Th1 cells that secrete IFN-γ, and Th1/Th2 cell imbalance shifts to the predominance of Th1 cell drift. The imbalance of Th1/Th2 cell population activates the pathological immune response of the thyroid, leading to a series of pathophysiological changes (20).

The expression levels of IFN-γ, IL-2, IL-4, IL-6 and IL-10 in serum of HT group and control group were detected. It was found that the levels of IFN-γ, IL-2 and IL-6 in the serum of the HT group were higher than those of the control group, while the levels of IL-4 and IL-10 were lower.

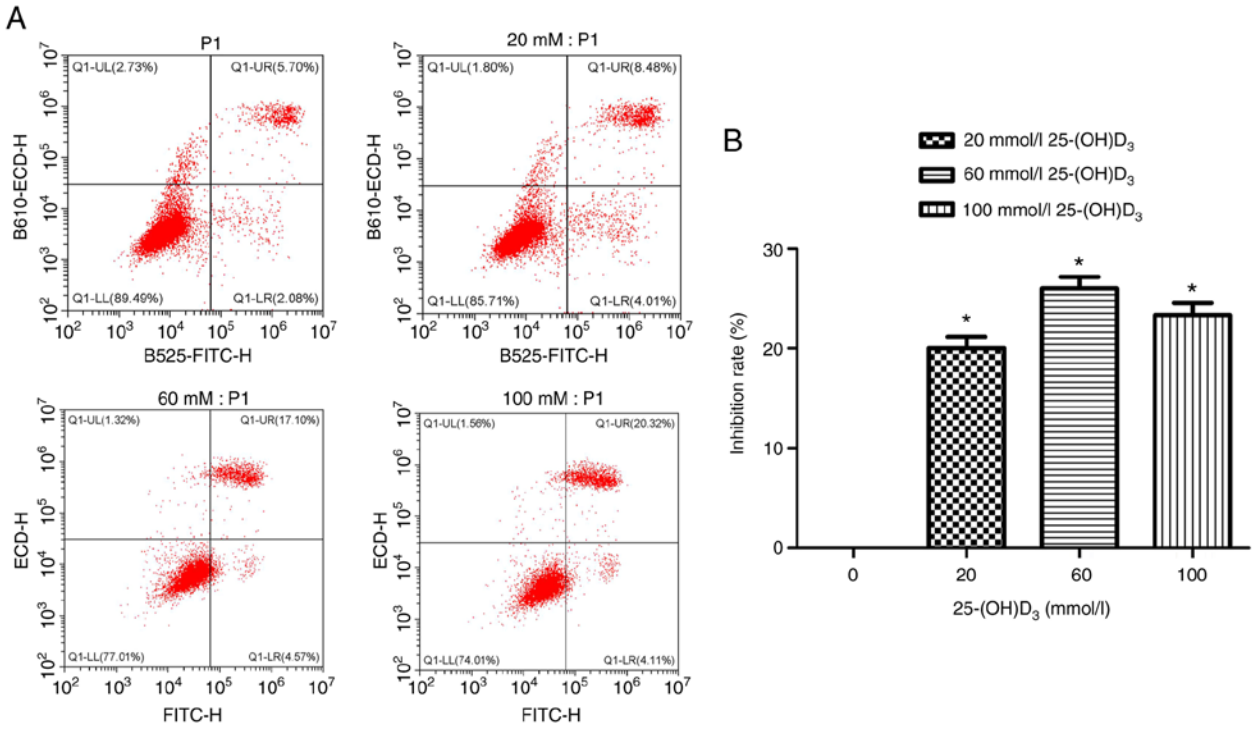

Figure 3. Effects of different concentrations of 25-(OH)D_3 on the apoptosis of Nthy-ori3-1 cells. (A) Representative flow cytometry chart of Nthy-ori3-1 cell apoptosis after treatment with different concentrations of 25-(OH)D_3. (B) Semi-quantitative analysis of apoptosis after treatment with different concentrations of 25-(OH)D_3. *P<0.05 vs. the untreated control group.

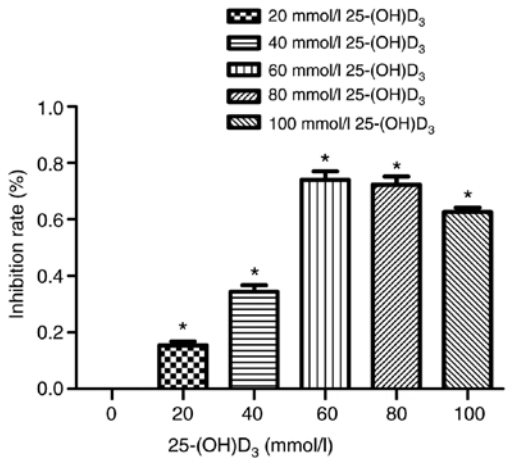

Figure 4. Effects of different concentrations of 25-(OH)D_3 on the proliferation of Nthy-ori3-1 cells. Nthy-ori 3-1 thyroid cells were treated with different concentrations of 25-(OH)D_3 (0, 20, 40, 60, 80, 100 mmol/l) and cell proliferation was detected. *P<0.05 vs. the untreated control group.

Vitamin D is an important vitamin necessary for the human body. With the discovery of its vitamin D receptor (VDR) in many immune organs and tissues, it was found that it plays an important role in immune regulation. Studies have shown that HT patients may have 25-(OH)D_3 deficiency (21). It was previously found that the level of 25-(OH)D_3 was significantly correlated with the levels of IL-2, IFN-γ and IL-4 in patients with HT hypothyroidism, suggesting that 25-(OH)D_3 could affect the immune function of the body by affecting the secretion of Th1 and Th2 cytokines (22). The degree and incidence of the disease can be reduced by adding 25-(OH)D_3 to experimental autoimmune thyroiditis (AIT) rats and giving 25-(OH)D_3 before the onset of AIT. After the application of 25-(OH)D_3, the levels of IFN-γ and IL-12 were found to be decreased, while the levels of IL-4 and IL-10 were increased (23). There are few reports concerning the correlation between 25-(OH)D_3 and Th1/Th2 cytokines in patients with HT. This study showed that the level of 25(-OH)D_3 was negatively correlated with the levels of IL-2, IFN-γ, IL-4, IL-6 and IL-10 in the serum of HT patients, which is consistent with existing research results. It suggested that serum 25-(OH)D_3 levels in HT patients may be involved in disease progression by upregulation of IFN-γ and IL-2 levels and downregulation of IL-10 levels.

Autophagy is a function that allows cells to maintain cell activity by degrading and recycling harmful substances such as damaged organelles through lysosomes. Mammalian target of rapamycin (mTOR) is a conserved protein kinase that plays a key role in coordinating the balance between cell growth and autophagy. When cells lack nutrients, mTOR can be inhibited to induce autophagy. mTOR and LC3B are two important autophagy-related proteins. mTOR-mediated signal transduction acts on downstream effectors, which can initiate transcription and translation of related genes and regulate autophagy. Activation of the mTOR signaling pathway can inhibit autophagy, and LC3B is an important marker of autophagy. When autophagy is activated, cytoplasmic LC3B (i.e., LC3B-I) changes into membrane LC3B (i.e., LC3B-II). Its expression level can be used as an indicator to evaluate the level of autophagy.

In the present study, the high expression of mTOR protein and the low expression of LC3B-II protein in thyroid tissues of patients with HT revealed that the level of autophagy in

HT tissues was lower than that in normal tissues. Nthy-ori3-1 thyroid cells were treated with different concentrations (0, 20, 40, 60, 80 and 100 mmol/l) of 25-$(OH)D_3$ for 24 h. Western blot results showed that with the increase in concentration, the expression level of mTOR protein increased, and the expression level of LC3B-II protein was decreased, especially when the concentration of 25-$(OH)D_3$ was 60 mmol/l. This suggested that a certain concentration of 25-$(OH)D_3$ inhibited the expression of autophagy-related protein LC3B-II by raising mTOR. Consistently, the level of autophagy-related protein LC3B-II in the thyroid tissue of HT patients was lower than that in normal tissues, suggesting that in HT patients, 25-$(OH)D_3$ induces abnormal autophagy in thyroid epithelial cells and is related to HT. With the increase of 25-$(OH)D_3$ concentration, the cell proliferation ability obviously decreased. A certain concentration of 25-$(OH)D_3$ can participate in the process of HT by inhibiting autophagy and proliferation of thyroid epithelial cells.

In conclusion, the present study explored the relationship of Th1 and Th2 cytokine balance with 25-$(OH)D_3$ in HT patients. Human HT were selected to detect the effect of 25-$(OH)D_3$ on autophagy and proliferation of human HT. The mechanism of vitamin D on HT at cellular level was discussed. This study provides a new direction and idea for the prevention, control and treatment of Hashimoto's thyroiditis.

Acknowledgements

Not applicable.

Funding

This work was supported by Qiqihar Science and Technology Plan Project (SFGG-201940).

Availability of data and materials

The datasets used and/or analyzed during the present study are available from the corresponding author on reasonable request.

Authors' contributions

JH, YL, HL, CZ, JZ, XS and SZ conceived and designed this study. JZ offered administrative support. JH, YL, HL, CZ, JZ, XS and SZ dealt with the experimental materials for study. JH, YL, HL, CZ, JZ and XS helped with data collection and summary. JH, YL, HL, CZ, JZ, XS and SZ were responsible for data analysis and interpretation. JH, YL and JZ wrote the manuscript. All authors read and approved the final manuscript.

Ethics approval and consent to participate

The study was approved by the Ethics Committee of the Second Affiliated Hospital of Qiqihar Medical University. Signed written informed consents were obtained from the patients.

Patent consent for publication

Not applicable.

Competing interests

The authors declare that they have no competing interests.

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