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Von Willebrand Factor, and Soluble Intercellular and Vascular Cell Adhesion Molecules as Indices of Endothelial Activation in Patients with Congenital Hypothyroidism

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Key Words
Congenital hypothyroidism · Von Willebrand factor · Endothelial function · Vascular cell adhesion molecule · Intercellular cell adhesion molecule

Abstract
Considering the high prevalence of congenital hypothyroidism (CH) in Isfahan, the possible involvement of endothelial dysfunction in the pathogenesis of CH and the lack of studies in this field, the aim of this study was to determine the endothelial function in CH patients. ***Methods:*** During this case-control study, the endothelial function in CH neonates and in those with normal screening results was evaluated during a CH screening program in Isfahan. Peripheral blood samples were obtained for measurement of the von Willebrand factor (vWf), and intercellular and vascular cell adhesion molecule (ICAM and VCAM). In CH patients these biomarkers were measured twice: before and 4 weeks after treatment. ***Results:*** In this study, 56 neonates were evaluated: 30 of them were neonates with normal screening results and 26 were diagnosed with CH and classified into two groups according to their TSH levels. The mean ICAM and VCAM were higher in CH patients than in the control group ($p < 0.05$). The mean ICAM and VCAM decreased significantly after treatment in CH patients ($p < 0.05$). There is no significant relationship between TSH, ICAM, VCAM and vWf ($p > 0.05$). ***Conclusion:*** The findings of this study demonstrate the possible involvement of the endothelial system in the pathogenesis of CH and its cardiovascular complications. Further studies with a larger sample size and with the measurement of other endothelial function markers are needed.

Introduction

Congenital hypothyroidism (CH) is the most common endocrine disorder with an incidence of 1/3,000–4,000 live births worldwide [1]. It is characterized by an insufficient production of thyroid hormone due to thyroid dysgenesis or dyshormonogenesis [2]. Although the implementation of screening of newborns for CH during the past decades has resulted in a significant improvement in eliminating its related long-term sequels such as mental retardation and many other neurodevelopmental anom-

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alies, many studies have demonstrated that persistent selective impairments such as cardiovascular abnormalities may still occur in these children [3].

Recent studies have demonstrated that CH patients display cardiovascular abnormalities such as impairment of left and right ventricular systolic and diastolic function, reduction of exercise capacity and cardiopulmonary performance and increase in carotid intima-media thickness despite an appropriate replacement therapy [4–6].

It has been shown that regardless of its indirect antiatherosclerotic effect by modifying atherosclerosis risk factors such as hyperlipidemia, the thyroid hormone has direct vascular effects through production of some vasodilator and vasculoprotective molecules [7]. Therefore, these associations provide an additional link between hypothyroidism, vascular disease and endothelial dysfunction. However, the precise mechanisms responsible for these findings are not completely understood but a role of the thyroid hormone through its direct effect on the vascular and endothelial function has been proposed [8, 9]. The association between the mentioned cardiovascular abnormalities, especially increased intima-media thickness, supports the assumption of endothelial involvement in thyroid disorders [10]. Endothelial involvement in disease can vary from activation and dysfunction to damage and injury [11]. Several methods can be used to measure endothelial function. Flow-mediated dilation measured by ultrasound reflects the endothelial production of NO, but is difficult to standardize and use in clinical practice. Several biological markers reflect endothelial activation or injury such as von Willebrand factor (vWF), and the soluble form of intercellular and vascular cell adhesion molecules (ICAM and VCAM) [12].

vWF is an endothelial-specific ligand for platelet glycoproteins which plays a pivotal role in mediating platelet adhesion to damaged arterial walls [12–15]. When endothelial cells (ECs) are injured, vWF is released from endothelial Weibel-Palade bodies [14]. vWF has been considered a gold standard in the measurement of endothelial damage and is increased in several disease states associated with endothelial dysfunction [14]. The plasma level of vWF is predictive of cardiovascular events in healthy subjects [14–16].

ICAM and VCAM are endothelial ligands for integrins expressed on leukocytes and platelets which facilitate endothelial adhesion of circulating leukocytes. The expression of ICAM-1 and VCAM-1 is increased in response to inflammatory states and predicts vascular events in healthy individuals [16].

Considering the high prevalence of CH in Isfahan [17] and the importance of proper management of CH patients in order to prevent its related short- and long-term consequences such as cardiovascular disease and atherosclerosis, the possible involvement of endothelium in the pathogenesis of CH and the lack of studies in this field, the aim of our study was to assess endothelial function in CH patients by measuring ICAM, VCAM and vWF. Moreover, this is the first study in the field to investigate the effect of levothyroxine treatment on endothelial function. However, previous studies indicated that measuring these markers is not invasive and their expression is greater in children than in adults [18].

Patients and Methods

In this case-control study, endothelial function in CH neonates and those with normal screening results was evaluated during a CH screening program in Isfahan, Iran, from February 2008 to May 2010.

According to the guidelines of the CH screening program, 3- to 7-day-old neonates with TSH >10 mIU/l using filter paper were recalled and reexamined and those with abnormal T_4 and TSH level on their second measurement (TSH >10 mIU/l and T_4 <6.5 μg/dl) were diagnosed as CH patients and received treatment and regular follow-up. Levothyroxine was prescribed for hypothyroid neonates at a dose of 10–15 μg/kg/day as soon as the diagnosis was confirmed, with the monitoring of TSH and T_4 every 1–2 months during the first year of life and every 1–3 months during the second and third years. Patients made frequent visits to ensure appropriate T_4 and TSH concentration [19].

The population studied consisted of CH patients and a group of neonates with normal screening results representing the normal control group.

The population studied was selected by convenience method from CH patients referred to the Isfahan Endocrine and Metabolism Research Center for treatment and follow-up and also from neonates with normal screening results. CH patients included those with serum TSH levels (second measurement after recall) of 10–50 mIU/l (group 1) and >50 mIU/l (group 2). CH patients with a history of septicemia, immaturity and confirmed metabolic disorder were excluded from the study.

Written consent was obtained from the parents of the CH patients. The protocol was approved by the Institutional Review Board and Medical Ethics Committee of the Isfahan University of Medical Sciences.

Baseline characteristics of the participants were obtained from their screening questionnaire. Complementary information about CH patients was obtained from their registered profiles in the Endocrinology and Metabolism Research Center. Peripheral blood samples were obtained from the population in which vWf, ICAM and VCAM were measured. In CH patients these biomarkers were measured twice: before treatment and 4 weeks after treatment.

For the measurement of vWf, ICAM and VCAM, 1 ml of plasma was obtained by centrifuging the blood sample and it was stored at –70°C until assayed.

Table 1. Baseline characteristics of the CH patients and neonates with normal screening results (control group)

	CH patients (n = 26)	Control group (n = 30)	p value
Age, days	16.2 ± 6.5	12.6 ± 5.3	NS
Female/male	14/13	15/15	NS
Weight, g	3,139.2 ± 527.1	3,388.2 ± 371.1	NS
Length, cm	50.1 ± 1.6	50.9 ± 2.2	NS
Head circumference, cm	35.7 ± 1.4	34.7 ± 1.4	NS
Screening TSH, mIU/l	66.3 ± 7.6	5.41 ± 2.64	0.00
T_4, μg/dl	3.9 ± 0.97	8.7 ± 2.2	0.00
vWf, %	51.2 ± 6.9	42.5 ± 4.2	NS
ICAM, nmol/l	39.5 ± 4.0	23.3 ± 4.6	0.01
VCAM, nmol/l	56.6 ± 4.7	39.2 ± 5.7	0.02

NS = Nonsignificant.

Table 2. Endothelial markers studied (mean ± SD) in the two CH groups (group 1: TSH 10–50 mIU/l, n = 10 and group 2: TSH >50 mIU/l, n = 16) before and after treatment

	Before treatment	After treatment	p value
vWf, %			
Group 1	50.9 ± 12.2	58.1 ± 23.3	NS
Group 2	51.5 ± 8.3	31.8 ± 6.2	NS
ICAM, nmol/l			
Group 1	33.8 ± 13.6	26.5 ± 7.1	NS
Group 2	39.3 ± 6.7	28.3 ± 2.6	0.02
VCAM, nmol/l			
Group 1	58.7 ± 14.0	25.9 ± 7.1	0.00
Group 2	52.9 ± 7.1	34.2 ± 3.9	0.005

NS = Nonsignificant.

Laboratory Measurement

Plasma vWF was measured by sandwich enzyme-linked immunosorbent assay (ELISA), using kits from Diagnostica Stago (Asnières, France) and plasma levels of ICAM and VCAM were measured with the ELISA method, using kits from Bender MedSystems GmbH) (Vienna, Austria). Serum T_4 and TSH concentrations were measured by radioimmunoassay and immunoradiometric assay, respectively, using kits from Kavoshyar (Tehran, Iran). Thyroid function tests were performed by Berthold LB 2111 unit gamma counter equipment.

Statistical Analysis

Data were analyzed using the SPSS statistical software ver. 13 (SPSS Inc., Chicago, Ill., USA). Descriptive data are expressed as mean ± standard deviation. Quantitative data of the 2 groups and before and after treatment in the group of CH patients were compared using the Student t test or paired t test. $p < 0.05$ was considered statistically significant.

Results

Fifty-six neonates were studied: 30 of them as neonates with normal screening results and 26 with diagnosed CH in 2 different groups according to their TSH levels. Baseline characteristics of the studied neonates in case and control groups are represented in table 1.

Mean ± standard deviation of studied endothelial markers in the two studied CH groups (group 1: TSH 10–50 mIU/l and group 2: TSH >50 mIU/l) before and after treatment is presented in table 2.

The mean of the studied endothelial markers among all studied CH patients before and after treatment was significantly different for ICAM ($p = 0.01$) and VCAM ($p = 0.000$) and it tended to be significant for vWF ($p = 0.07$). There was no significant association between TSH or T_4 and the markers ($p > 0.05$).

Discussion

To our knowledge, this is the first study which evaluated the endothelial activation in CH patients and the effect short-term levothyroxine treatment had on it. The results of our study indicate that adhesion molecules and vWF were significantly higher in CH patients than in normal controls and short-term levothyroxine treatment decreases these biomarkers in CH patients.

Endothelium is considered to be the key regulator of vascular tone and structure which balance the release of contracting and endothelial-derived relaxing factors in blood vessels [20]. It is involved in the pathogenesis of many diseases and can be considered as an important objective biological factor that has become the focus of recent research in order to assess new prevention and health promotion strategies [21].

When ECs are stressed, a change in the resting, constitutive phenotype of the endothelium to an 'activated' phenotype is induced, a change which is reversible. EC activation is characterized by an increased or de novo expression of leukocyte adhesion molecules, a change in phenotype from antithrombotic to prothrombotic, cytokine and growth factor production, and upregulation of HLA molecules [22, 23]. Retraction of stressed ECs re-

sults in exposing the subendothelial tissue and exocytosis of the Weibel-Palade body with subsequent surface expression of P-selectin and release of vWF into the plasma. Upregulation of leukocyte adhesion molecules such as selectins, ICAM-1 and VCAM-1 allows leukocytes to adhere to the endothelium and then move into the vascular tissues leading to neointima and fatty streak formation, which represent early stages of atherosclerosis [24, 25]. These events are reversible disorders, and therefore therapeutic intervention before development of irreversible vascular injury is extremely important [26, 27].

On the other hand, considering the high prevalence of CH in our region [17] and the necessity of planning a more appropriate and multidisciplinary screening program, identifying the relation between endothelial function and CH made us consider new aspects in screening and treating the disorder.

Current evidence supports the direct effects of thyroid hormones on the vascular and consequently on the endothelial function. It has an important role in the regulation of cardiovascular function [7].

There are several reports on endothelial dysfunction in hypothyroidism in the adult population [28, 29], but less information is available on CH patients [30] and there is no study in this field of CH patients during the neonatal period. In a recent study Oliviero et al. [30] investigated the effects of long-term levothyroxine replacement therapy on the endothelial function in young adults with CH and concluded that young CH patients treated with long-term levothyroxine therapy present endothelial dysfunction which was demonstrated by lower flow-mediated dilation and decreased vascular distensibility. According to their findings serum TSH level fluctuations during treatment and follow-up could decrease the vascular distensibility and activity. Although the method we used to evaluate the endothelial function was not similar to that of the study of Oliviero et al. and we evaluated the short-term effect of levothyroxine treatment on endothelial function, the findings of our study were similar to those of their study.

The plasma level of vWF was not different in hypothyroid and control groups [28]. Similarly, in this study the serum vWF concentration was not different among CH patients and the control group, it was not different in CH patients with different TSH levels and it was not significantly changed after treatment in CH patients. There was a trend toward a decreased serum level of vWf in CH patients after treatment ($p = 0.07$). However more studies with a larger sample size are needed for more accurate judgment.

The adhesion molecules ICAM and VCAM are considered as important biochemical markers for the low-grade vascular inflammation and endothelial dysfunction and recent studies have suggested that a detection of these markers early in life may be predictive for the development of endothelial dysfunction-related disorders, such as atherosclerosis [31]. In this study, the serum level of ICAM and VCAM in CH patients was significantly higher than in the control group; however, the mean of the markers decreased significantly after treatment to approximately the level in the control group. Serum levels of ICAM and VCAM were not significantly different in the two studied CH groups with different TSH levels.

In this study, there was no significant difference in serum levels of all studied endothelial markers among CH patients with different TSH levels and there was no significant relationship between serum TSH and the endothelial biomarkers. This may be due to the small sample size of studied patients in the two groups, the short duration of the study or it can in part be explained by the use of the methods for evaluating endothelial function.

Although many studies show that there is a relationship between endothelial function and TSH and that a higher level of TSH could predict the presence of endothelial dysfunction, there are studies which did not report a similar correlation [23, 32]. Gottardi et al. [32] studied the relationship between TSH and endothelial function in children. According to their findings, endothelial function does not seem to be correlated with TSH. As other studies indicated, these findings suggest that mechanisms other than hypothyroidism, such as low-grade inflammation and autoimmune factors, could play a role in the endothelial function in CH patients. Hypothyroidism is considered to be the manifestation of a worsening condition; therefore, prominent endothelial dysfunction assessed by different methods may be observed after levothyroxine treatment and during long-term follow-up [33, 34].

The findings of this study demonstrated the possible involvement of the endothelial system in the pathogenesis of CH and its cardiovascular complications. It seems that further studies with a larger sample size during the follow-up period and with the measurement of other endothelial function markers are needed.

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