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Progress in Research on Nonalcoholic Fatty Liver and Gestational Diabetes

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Abstract

Although there is ample evidence that non-alcoholic fatty liver disease (NAFLD) is associated with impaired glucose homeostasis in the body, the clinical significance of NAFLD in pregnant women has not been established. Current studies have shown that women with NAFLD during early pregnancy have a significantly increased incidence of gestational diabetes mellitus (GDM) during pregnancy; whereas women with a history of GDM have a significantly increased probability of developing NAFLD in the future. Both may be a manifestation of an etiology in both systems, reflecting the impaired glucose homeostasis and the continuity of insulin resistance. For women with NAFLD found in early pregnancy, it is recommended to closely monitor blood glucose during pregnancy, and if necessary, early intervention to strengthen prenatal and postnatal care. The presence of GDM at a young age in women may be an early marker that helps to screen out women at higher risk of developing a disease before significant metabolic disease, and is of great significance in reducing associated morbidity and mortality.

Keywords

Nonalcoholic Fatty Liver Disease, Gestational Diabetes Mellitus, Tumor Necrosis Factor, Leptin, Estrogen

1. Introduction

Non-alcoholic fatty liver disease (NAFLD) refers to a clinical syndrome characterized by the exclusion of hepatocyte fat accumulation caused by alcohol and other factors that are clearly damaging to the liver. It is a liver damage caused by insulin resistance and genetically related metabolic stress, including nonalcoholic fatty liver disease (NAFLD), steatohepatitis nonalcoholic, cirrhosis and liver cancer [1] [2]. In Western countries, 20% - 30% of people suffer from NAFLD

[3]. A recent study [4] showed that a quarter of Asian populations have NAFLD. A study published in 2016 showed that the prevalence of NAFLD in seven provinces and cities in China was 43.3% [5]. Clinical Diagnostic Criteria: Any of the following items 1 - 5 and 6 or 7 can be diagnosed as NAFLD. 1) Risk factors: obesity, type 2 diabetes, hyperlipidemia, etc.; 2) No history of alcohol consumption or alcohol consumption. Males < 140 g per week, women < 70 g per week; 3) Excluding viral hepatitis, drug-based liver disease, total parenteral nutrition, hepatolenticular degeneration and autoimmune liver disease can lead to specific diseases of fatty liver; 4) In addition to the clinical manifestations of the primary disease, there may be fatigue, liver pain, liver spleen, etc., symptoms and signs; 5) Serum transaminases or γ-GT, transferrin increased; 6) In line with the diagnostic criteria of fatty liver disease; 7) Liver histological changes in line with the pathological diagnostic criteria for fatty liver disease. NAFLD is closely related to obesity, type 2 diabetes, cardiovascular disease, etc., and its prevalence is also increasing [6] [7]. It is an increasingly common cause of cirrhosis and hepatocellular carcinoma and is becoming the most common indication for liver transplantation in the United States. Given the increasing prevalence and burden of disease in NAFLD, it is important to identify patients with NAFLD before advanced liver disease.

Gestational diabetes mellitus (GDM) is a common pregnancy complication that often manifests as spontaneous hyperglycemia that occurs during pregnancy [8]. Blood glucose levels in most patients with GDM return to normal after delivery, but GDM has an adverse effect on pregnancy outcomes and may have an impact on the long-term health of mothers and infants, including the mother's increased risk of developing type 2 diabetes and cardiovascular disease in the future, as well as the risk of future obesity, cardiovascular disease, type 2 diabetes and/or GDM in children [9]. The latest relevant literature reports mentioned that the prevalence of GDM in the United States was 7.6%, and 19.7% of women were diagnosed with diabetes at subsequent follow-up [10]. The incidence of GDM in China is 1% - 5%, and there has been a significant increase in recent years.

Although there is ample evidence that non-alcoholic fatty liver disease (NAFLD) is associated with impaired glucose homeostasis in the body, the clinical significance of NAFLD in pregnant women has not been established. More and more studies have confirmed the close relationship between NAFLD and GDM, and the two interact with each other. This paper describes the research progress of the correlation between NAFLD and GDM.

2. Clinical Relevance of NAFLD and GDM

2.1. Women with NAFLD during Early Pregnancy Have an Increased Probability of Developing GDM during Pregnancy

De Souza LR *et al.* [11] conducted a prospective cohort study in a clinic at a large obstetrics and gynecology hospital in Toronto: 476 pregnant women participated

in the study and assessed whether participants were assessed by ultrasound at 11 - 14 weeks of gestation With NAFLD, all study participants were tested for GDM by oral glucose tolerance test (OGTT) after 8 hours of overnight fasting at 24 - 28 weeks of gestation. De Souza LR believes that according to the examination, it is confirmed that women with NAFLD during early pregnancy can predict the blood glucose status of pregnant women in the middle and late pregnancy.

Seung Mi Lee *et al.* [11] recruited 678 women in the 14th week of pregnancy at the In Seoul Women's Hospital in Incheon and the Boramae Medical Center in Seoul National University. After screening, 608 women participated in the experiment. The results of the experiment showed that pregnant women with nonalcoholic fatty liver disease were more likely to develop GDM in the third trimester of pregnancy at 10 - 14 weeks, and the risk of developing GDM increased gradually according to the severity of NAFLD: grade 0 fat The incidence of GDM in degenerative women was 3.2%, 10.5% in patients with grade 1 steatosis, and 42.3% in patients with grade 2 steatosis.

The clinical practice guidelines recommend GDM screening at 24 - 28 weeks of gestation, but this may be too late and is not conducive to maternal and child outcomes. Fatty liver in early pregnancy suggests that the woman has insulin resistance (IR), a subclinical condition that may affect the health of pregnant and postpartum women [12]. Subtle anomalies in glucose homeostasis may also indicate pre-diabetes status during pregnancy [13]. Therefore, it is important to consider the changes in blood glucose found in women with NAFLD in early pregnancy in order to intervene in advance.

2.2. Increased Prevalence of NAFLD in Women with Previous GDM

Hypertriglyceridemia and high insulin levels caused by physiological stress during pregnancy may contribute to women who are at high risk of developing NAFLD [14]. Studies have shown that women with a history of GDM and a history of postpartum weight retention have increased systemic inflammatory responses and reduced insulin sensitivity [15]. In a cohort of Ajmera VH *et al.* [16] from a multidisciplinary coronary risk development in adolescents in the United States, 1115 women who had been pregnant were followed up for 25 years and found that in the next 25 years, there was GDM. Women with a history of disease were more than twice as likely to develop NAFLD as women without a history of GDM. This study demonstrates that the history of GDM is a risk marker for NAFLD in middle-aged women, suggesting opportunities to identify women at higher risk of developing a higher risk.

3. NAFLD and GDM Are Related to Insulin Resistance

Clinical studies have found that serum insulin levels and insulin resistance index are significantly elevated in patients with NAFLD, indicating insulin resistance in patients with NAFLD [17]. The more popular "second strike" theory holds that the fat caused by insulin resistance accumulates in the liver and is the first

blow; then oxidative stress and lipid peroxidation damage are based on this, which is the second blow, and finally leads to the liver inflammation [18]. Moreover, due to the persistence of steatohepatitis, a vicious circle of "inflammation-necrosis-inflammation" is finally formed [19].

When women's gestational age gradually increases, the nutritional needs of the fetus will increase, and the fasting blood glucose will be lower in the early pregnancy, which is about 10% lower than usual. In the middle and late pregnancy, with the increase of antagonistic insulin-like substances, in order to maintain the normal level of glucose metabolism, the insulin demand will increase accordingly. If the mother's insulin secretion is limited, GDM will appear [15].

3.1. Insulin Resistance Is an Important Factor in the Development of NAFLD

Insulin resistance leads to hyperinsulinemia and high levels of free fatty acids (FFA) in the blood, and hyperinsulinemia can increase insulin resistance as well as increased FFA. FFA can produce fat, and the activity of lipoprotein lipase and the synthesis of fat are decreased when insulin resistance is enhanced, resulting in an increase in FFA in the blood. The FFA enters the liver and leads to an increase in the synthesis of triglycerides. Increased triglycerides bind to apolipoproteins to form very low density lipoproteins [20]. Therefore, when the formation of triglyceride is greater than the output, lipid accumulation is formed in the liver, and finally fatty liver is gradually formed. When too much lipid is stored in the liver cells, it will damage the insulin receptor on the cell membrane, resulting in reduced reactivity and sensitivity to insulin [21]. It has been reported that patients with hypertriglyceridemia have increased serum FFA, interfere with insulin-receptor binding, decrease insulin sensitivity, produce insulin resistance, and high levels of serum FFA levels can inhibit insulin signaling [22]. At the same time, hyperinsulinemia leads to an increase in fatty acid synthesis, further enhancing the accumulation of lipids in the liver.

3.2. IR Plays a Key Role in the Pathogenesis of GDM

With the development of pregnancy, the mother antagonizes the increase of insulin-like substances, such as: leptin, FFA, tumor necrosis factor-α (TNF-α), high levels of estrogen, prolactin, cortisol, Placenta prolactin and so on. TNF-[alpha] is a polypeptide produced by activated monocytes-macrophages. The placenta and uterine decidua contain a significant amount of macrophages, which, when stimulated by fetal antigens, activate to produce large amounts of TNF-α [23]. High levels of TNF-α in the blood reduce the degree of tyrosine phosphorylation of the insulin receptor and attenuate insulin signaling, leading to IR [24]. Increases the FFA in the blood, and then increases the expression of TNF-α through the lysosomal pathway, causing mitochondrial structure and function abnormalities in liver cells, oxidative stress, and fatty acid β oxidation overload, eventually leading to fat deposition in the liver [25] [26].

Leptin is a fat-soluble hormone synthesized and secreted by fat cells. During pregnancy, leptin levels increase, and oxidative stress is promoted by accumulation of reactive oxygen species, which induces islet β cell damage, which is closely related to the pathogenesis of GDM [27] [28]. In theory, leptin can strengthen fat breakdown and reduce synthesis. In fact, the study found that in patients with fatty liver, the level of leptin is increased, considering the presence of "leptin resistance" in patients with NAFLD [29]. Some scholars believe that there may be a "fat-insulin endocrine axis" between fat and islets, and a two-way feedback loop is formed between leptin and insulin through fat and islets [30]. Under normal circumstances, leptin promotes fat breakdown and inhibits islet secretion of insulin. In patients with NAFLD, due to leptin resistance, fat breakdown is limited, leptin inhibits the ability of insulin secretion from islets, leading to hyperinsulinemia, aggravating IR, and accumulation of fat in the liver [31] [32]. TNF-α and leptin play an important role in insulin resistance, eventually leading to the occurrence of NAFLD.

Estrogen at physiological concentrations has the effect of promoting insulin expression and increasing the body's sensitivity to insulin [33]. During pregnancy, the body's estrogen level is significantly increased, reaching a peak at the end of pregnancy. At this moment, the level of estriol is 1000 times that of non-pregnant women, and the levels of estradiol and progesterone are 100 times that of non-pregnant women. High concentrations of estrogen reduce insulin sensitivity by affecting insulin-like receptor expression, leading to IR [34] [35].

Glucocorticoids can inhibit the glycemic uptake function of insulin [36], which alters the receptors of insulin by transmembrane translocation of glucose, which may be the main target of diabetes [37]. Moreover, glucocorticoids have an "allowed effect" on progesterone and are indirectly related to IR.

NAFLD and GDM are related to insulin resistance. The diagnosis of NAFLD in pregnancy suggests that the pregnant woman may already have insulin resistance early or even before pregnancy. Combined with the physiological state of women during pregnancy, early insulin resistance promotes the development of GDM in the third trimester of pregnancy. GDM during pregnancy represents a state of pancreatic β-cell dysfunction, especially when glucose post-load increases. Previous studies have shown that women with a history of GDM and a history of postpartum weight retention may increase systemic inflammation and reduce insulin sensitivity, which may be associated with an increased probability of developing NAFLD in women with a history of GDM [36].

4. Summary

In summary, women with NAFLD during early pregnancy have a significantly increased incidence of GDM during pregnancy; whereas women with a previous history of GDM have a significantly increased probability of developing NAFLD in the future. The above discussion reflects the impaired glucose homeostasis and the continuity of IR, which may be the cause of an etiology in both systems.

The two may also have a relationship that promotes a vicious circle of chains. Currently, there are no guidelines on what populations should be screened for NAFLD, and some social guidelines specifically recommend not screening for NAFLD [37]. However, this may change as the number of people treating NAFLD increases and the screening method improves. For women with NAFLD found in early pregnancy, it is recommended to closely monitor blood glucose during pregnancy, and if necessary, early intervention to strengthen prenatal and postnatal care. A female GDM history may be an early marker that can screen young women who may have NAFLD in the future before a significant metabolic disease, and can be targeted to prevent NAFLD-related diseases.

Conflicts of Interest

The authors declare no conflicts of interest regarding the publication of this paper.

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Assessment of Glycosaminoglycan Content of Lumbar Intervertebral Discs in Patients with Radiculopathy

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Abstract

Objective: To assess glycosaminoglycan (GAG) content of lumbar intervertebral discs (IVDs) in patients with radiculopathy compared with healthy volunteers with glycosaminoglycan chemical exchange saturation transfer (gagCEST). *Methods*: The lumbar spines of 15 patients with radiculopathy (9 women, 6 men; mean age 45 years; range: 19 - 80 years) and 13 healthy controls (10 women, 3 men; mean age 29 years; range: 19 - 38 years) without lumbar back pain or previous spine surgery were examined at a 3 Tesla (T) magnetic resonance imaging (MRI) scanner in this prospective study. The MRI protocol included standard morphological, sagittal, and transverse T2-weighted (T2w) images of the five lumbar IVDs (L1-S1) to assess Pfirrmann score and to detect disc disorders according to the Combined Task Force classification. To analyze biochemically the lumbar IVDs, a gagCEST sequence was applied to measure the GAG content of the nucleus pulposus (NP) and annulus fibrosus (AF). *Results*: Patients with radiculopathy indicated significantly lower gagCEST values in NP than healthy volunteers (2.82% ± 3.12% vs. 4.09% ± 2.25%, $P = 0.017$). The GAG content of AF showed no significant difference between volunteers and patients (2.66% ± 2.01% vs. 1.92% ± 2.56%; $P = 0.175$). *Conclusions.* Patients with radiculopathy presented with lower GAG values than healthy volunteers in NP, indicating an association between pain and IVD degeneration. gagCEST of lumbar IVDs is a powerful, non-invasive tool to investigate early disc degeneration, which we could demonstrate in the NP in our study collective.

Keywords

Glycosaminoglycan Chemical Exchange Saturation Transfer, Radiculopathy, Intervertebral Disc, Lumbar Spine, Early Degeneration

*Both authors contributed equally.

1. Introduction

Low back pain (LBP) is a common disease in the industrialized world with a high lifetime prevalence [1]. It is one of the leading causes of disability and imposes a high socio-economic burden [2]. Intervertebral disc (IVD) degeneration is one of the recognized causes of lower back pain [3]. IVDs consist of an outer annulus fibrosus (AF) and an inner nucleus pulposus (NP). Large proteoglycan molecules with numerous negatively charged glycosaminoglycan (GAG) side chains are a major component of IVDs, especially of the NP, which lead to a high osmotic pressure within the disc providing resistance to compressive loading [2] [4].

Magnetic resonance imaging (MRI) is well established in the assessment of IVD degeneration [5]. On T2-weighted (T2w) MR images, normal, non-degenerated intervertebral discs show a bright signal from the nucleus pulposus and inner fibers of the annulus due to a high amount of water [3] [5] [6]. Degenerative disc alterations can be visualized by a decrease in the water content on T2w images and be morphologically graded according to the Pfirrmann classification system [7]. Due to the degenerative process, the NP loses its translucency and becomes more difficult to distinguish from the surrounding AF [5]. Besides the Pfirrmann classification, IVDs can be graded according to the Combined Task Force (CTF) classification in normal appearance, protrusion, and extrusion of IVDs [8] [9].

Several biochemical MRI techniques have been used to assess and quantify extracellular matrix components of fibrous and hyaline cartilage, such as delayed gadolinium-enhanced MRI of cartilage (dGEMRIC), sodium MRI and T1 rho mapping to visualize the GAG content, and T2/T2* mapping to visualize collagen structure [10] [11] [12] [13] [14]. One relatively new and promising technique is glycosaminoglycan chemical exchange saturation transfer (gagCEST) [15]. Without the application of a contrast agent or dedicated additional MRI hardware, CEST imaging allows the determination of GAG content in IVDs [16] [17]. gagCEST of the lumbar spine is possible at a magnetic field strength of 3T. For gagCEST imaging, several images are acquired with presaturation pulses at different offset frequencies around the bulk water resonance and one reference image without saturation. The residual signal normalized to the reference image as a function of the offset frequencies (z-spectrum) can be utilized to determine and quantify the CEST effect according to magnetization transfer asymmetry ratio (MTRasym) values with respect to the water resonance due to the OH protons of GAG appearing in a frequency range of 0.9 to 1.9 ppm from the water resonance. The magnitude of the measured MTRasym values correlates directly with the underlying concentration of GAG [18] [19].

Haneder *et al.* have already shown a significant GAG loss in correlation with degenerative changes of IVDs in people with lower back pain [20]. Schleich *et al.* showed the same trend in a healthy collective [16]. The aim of our study was to compare the glycosaminoglycan content of the lumbar IVDs in patients with ra-

diculopathy with that of a healthy collective.

2. Materials and Methods

2.1. Hypothesis

Our hypothesis was that the gagCEST effect is lower in patients suffering from pain due to radiculopathy compared with healthy volunteers.

2.2. Study Population

The study was approved by the local ethics committee. Written informed consent was obtained from all volunteers for this prospective study. Fifteen volunteers with radiculopathy (9 women, 6 men; mean age: 45 years; range: 19 - 80 years) and 13 healthy volunteers (10 women, 3 men; mean age: 29 years; range: 19 - 38 years) without specific, subacute, and chronic low back pain or previous surgery of the lumbar spine were prospectively enrolled in this study. The diagnosis of radiculopathy originated from the doctor's letter or a clinical examination in our spine ambulance.

2.3. MR Hardware and Sequence Protocol

The lumbar spine of all participants was examined in supine position using a clinical whole-body 3T MR system (Magnetom Trio, A Tim System, Siemens Healthcare, Erlangen, Germany). Signal reception was performed using four channel body matrix coils and a 24-channel spine matrix coil. Our MR sequence protocol included a localizer and a T2w sequence in the sagittal and transverse orientations. Parameters of the sagittal T2w turbo spin echo sequence were as follows: field of view = 300 × 300 mm^2, basic resolution of 256 × 256, slice thickness = 3 mm, in-plane resolution = 1.2 × 1.2 mm, TR/TE = 3100/105 ms, number of slices = 15, flip angle = 160˚, two signal averages, number of echoes per slice = 17 and an acquisition time of 3 minutes and 39 seconds. The parameters of the transversal T2w turbo spin echo sequence were as follows: field of view = 240 × 240 mm^2, basic resolution of 384 × 307, slice thickness = 3 mm, in-plane resolution = 0.8 × 0.6 mm, TR/TE = 4000/113 ms, number of slices = 25, flip angle = 140˚, one signal average, number of echoes per slice = 26, and an acquisition time of 3 minutes and 38 seconds.

Biochemical imaging was performed using a prototype gagCEST and water saturation shift referencing (WASSR) sequences. CEST and WASSR sequences were composed of a presaturation module and a segmented 2D RF-spoiled gradient echo module. Detailed sequence parameters were given in **Table 1**.

CEST and WASSR images were motion-corrected using a diffeomorphic image registration approach incorporated in the prototype software fMRLung (Siemens Healthcare, Erlangen, Germany) [21]. The following data analysis was performed using in-house developed MATLAB software (The Mathworks, Inc., Natick, MA, R2012b). A reduction of image noise was performed using an in-plane 3 × 3 Gaussian filter. B0 field inhomogeneities were corrected using the

Table 1. Detailed sequence parameters of the gagCEST and WASSR sequence.

		WASSR	CEST
2D RF-spoiled GRE module			
T_E/T_R	[ms]/[ms]	5.56/575	3.01/1590
In-plane resolution	[mm^2]	0.8 × 0.8	0.8 × 0.8
Basic resolution		256 × 256	256 × 256
Slice thickness	[mm]	5	5
Flip angle	[˚]	12	12
Field of view	[mm^2]	150 × 150	150 × 150
Duration	[min:sec]	10:40	17:36
NEX (number of excitations)		2	2
Presaturation module			
Number of measured frequency offsets		42	26
Maximum frequency offset $\Delta\omega_{max}$	[ppm]	1	4
B1-CWAE	[μT]	1.5	0.3
Number of CEST presaturation pulses		1	8
PD/IPD	[ms]/[ms]	100/6	100/100

WASSR maximum symmetry algorithm [22]. The offset-corrected CESTcurves divided by the signal without CEST presaturation S0 are defined as z-spectrum Z(Ω). The magnetization transfer asymmetry was defined as MTRasym(DΩ) = Z(DΩ) Z(DΩ), where DΩ is the specified frequency shift difference. Evaluation of the gagCEST effect was determined using the MTRasym value in the frequency range from 0.9 to 1.9 ppm, which comprises the chemical exchange resonances of GAG hydroxyl protons [15].

2.4. Data Analysis

All lumbar IVDs (L1-S1; a total of 140 IVDs) could be imaged successfully without any dropouts. One radiologist with 6 years of experience in musculoskeletal radiology scored all lumbar intervertebral discs according to the Pfirrmann scoring system [7]. The scoring system is based on a five-step grading scale with grade 1 and 2 for non-degenerative discs and grade 3 - 5 for degenerative IVDs according to the nucleus signal intensity, the nucleus structure, the distinction between the nucleus pulposus (AP) and the annulus fibrosus (AF), and the disc height in midsagittal T2w images. According to the CTF classification, the same radiologist scored the IVDs into normal appearing discs and IVDs with protrusion or extrusion [9]. For both analyses, the radiologist was blinded to gagCEST values.

A region-of-interest (ROI) analysis was performed for MTRasym evaluation of the NP and AF to identify the gagCEST effect. All ROIs were selected by a self-acting image processing algorithm that detected the lumbar IVDs automati-

cally. This lumbar IVD segmentation was performed usingin-house developed MATLAB software. The disc segmentation was based on Bayes classification to divide bone and ligament from disc tissue of the lumbar spine. The segmentation area comprised the lumbar spine. According to the different tissue signal intensity of non-saturated and saturated images, the segmentation tool could distinguish IVDs from the other tissues of the lumbar spine by learning on several training objects before data analysis. The defined ROIs were divided into NP (the innermost 60% of the IVD) and AF (the remaining region of the IVD). Every automatically positioned ROI was visually checked by one radiologist with 6 years of experience in IVD segmentation. None of the ROIs were manually repositioned.

2.5. Statistical Analysis

SPSS (Version 22; SPSS; Chicago, IL) was used for statistical analysis. The mean, confidence intervals for the mean values, median, and standard deviations for the NP and AF were calculated as descriptive statistics. The Lilliefors test was used to verify the normal distribution. Because of the non-normally distributed data, we used Mann-Whitney U tests to compare gagCEST effects in NP and AF for patients and control groups. Results were considered statistically significant at $P < 0.05$.

3. Results

All completed measurements were technically successful. A total of 140 IVDs (L1-S1) of 15 patients with radiculopathy and 13 healthy volunteers were analyzed. Morphologically, 22 IVDs were scored Pfirrmann grade 1, 71 lumbar discs were scored Pfirrmann grade 2, 28 IVDs were graded Pfirrmann score 3, 18 discs were graded Pfirrmann grade 4, and 1 IVDwas scored Pfirrmann grade 5. Descriptive data are summarized in Table 2.

In non-degenerated (Pfirrmann grade 1-2) discs, significantly higher gagCEST values were found in the NP than in the AF ($P = 0.006$). In degenerated discs (Pfirrmann grade 3-5), no significant difference between the NP and AF could be detected ($P = 0.71$). We could demonstrate significantly lower gagCEST values in degenerated IVDs than in non-degenerated discs in the NP (2.18% ± 3.57% vs. 3.99% ± 2.1%, $P = 0.001$). For the AF, we found no significant difference between degenerated and non-degenerated IVDs. Lumbar discs without protrusion or extrusion showed significantly different gagCEST effects between the NP and AF ($P < 0.0001$). IVDs with protrusion or extrusion revealed no significant difference between the NP and AF ($P = 0.0923$; $P = 0.535$, respectively).

Patients with radiculopathy presented significantly lower gagCEST values in the NP compared withhealthy controls (2.82% ± 3.12% vs. 4.09% ± 2.25%, $P = 0.017$) (Figure 1, Figure 2). In the AF, no significant difference between patients and controls were found ($P = 0.175$).

Table 2. Descriptive data. Mean, standard deviation (Std), median, minimum, maximum, 95% confidence interval with lower and upper limit.

	Mean	Std	Median	Min	Max	CI [lower limit]	CI [upper limit]
AF total	2.27	2.35	2.44	−7.78	8.09	1.8811	2.6597
NP total	3.42	2.82	3.67	−10.21	12.11	2.9486	3.8827
AF Pfirr 1	1.87	1.96	1.54	−0.61	5.67	1.0500	2.6872
NP Pfirr 1	3.53	1.89	3.78	0.58	7.11	2.7429	4.3239
AF Pfirr 2	2.97	1.92	3.07	−1.60	8.09	2.5300	3.4131
NP Pfirr 2	4.14	2.28	4.14	−0.86	12.11	3.6169	4.6611
AF Pfirr 3	1.93	1.79	2.02	−2.17	6.29	1.2370	2.6146
NP Pfirr 3	2.93	2.18	2.75	−1.05	8.42	2.0985	3.7713
AF Pfirr 4	0.25	3.77	1.01	−7.78	5.22	−1.5388	2.0484
NP Pfirr 4	0.88	4.87	2.43	−10.21	6.51	−1.4414	3.1919
AF Pfirr 5	3.09	0	3.09	3.09	3.09	3.0943	3.0943
NP Pfirr 5	5.03	0	5.03	5.03	5.03	5.0314	5.0314
AF Pfirr 1+2	2.72	1.98	2.75	−1.60	8.09	2.3183	3.1139
NP Pfirr 1+2	4.00	2.20	3.99	−0.86	12.11	3.5567	4.4409
AF Pfirr 3-5	1.31	2.81	1.78	−7.78	6.29	0.4758	2.1377
NP Pfirr 3-5	2.19	3.60	2,67	−10.21	8.42	1.1300	3.2438
AF BSV 1	2.27	2.29	2.38	−7.78	8.09	1.8601	2.6797
NP BSV 1	3.44	2.69	3.65	−10.21	12.11	2.9553	3.9196
AF BSV 2	2.62	1.73	2.61	0.52	6.29	1.6396	3.5952
NP BSV 2	4.04	2.20	4.24	0.26	8.42	2.7948	5.2803
AF BSV 3	1.68	4.22	3.09	−7.03	5.22	−1.4480	4.7998
NP BSV 3	2.17	5.33	4.28	−9.16	6.51	−1.7833	6.1138
AF BSV 2+3	2.27	2.82	2.66	−7.03	6.29	1.0011	3.5400
NP BSV 2+3	3.35	3.64	4.28	−9.16	8.42	1.7092	4.9863
AF controls	2.66	2.01	2.52	−1.60	8.09	2.1734	3.1540
NP controls	4.09	2.25	4.01	−0.73	12.11	3.5433	4.6396
AF radiculopathy	1.92	2.56	2.28	−7.78	8.06	1.3480	2.5111
NP radiculopathy	2.82	3.12	3.38	−10.21	9.19	2.1218	3.5380

4. Discussion

Biochemical alterations of lumbar IVDs are present before morphological changes of the intervertebral disc appear [16] [19] [20]. GAG is one of the major components of the extracellular matrix of IVDs [5]. The loss of tissue water content of the IVD due to a depletion of GAGs plays a central role in these degenerative processes, at first in the NP [2] [4].

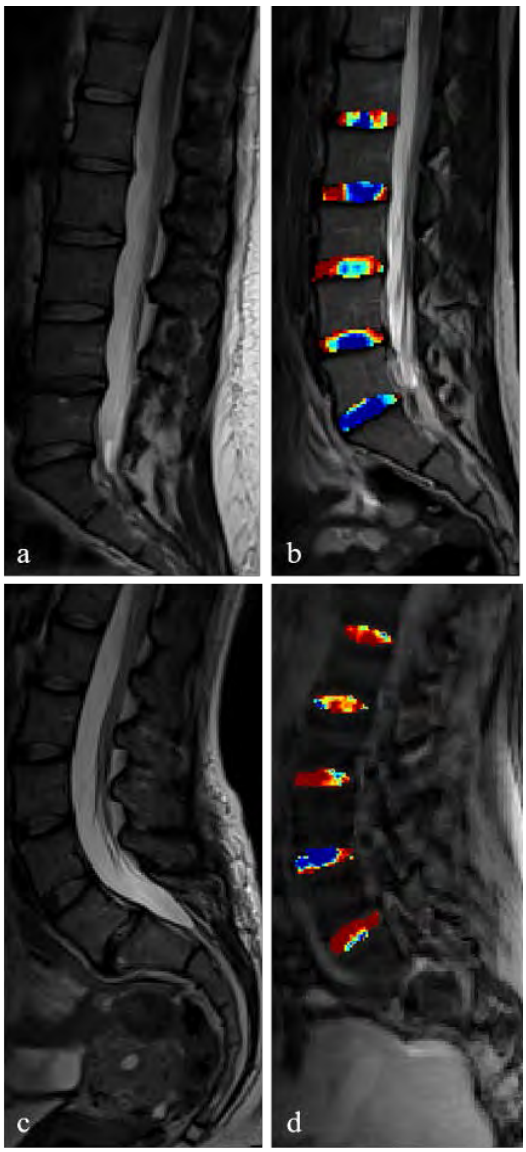

Figure 1. T2-weighted images (image (a), (c)) and color-coded gagCEST maps (image (b), (d)) of the lumbar spine (L1-S1). Morphological images of a healthy control (image (a)) and a patient suffering from radiculopathy (image (c)) revealed almost the same Pfirrmann grading. Biochemical images illustrated lower gagCEST values of patients with radiculopathy compared with healthy controls (image (b), (d)). Especially the motion segment of the affected nerve root (L5/S1) showed the lowest GAG values in this patient (image (d)).

Our results illustrated significantly lower gagCEST values in patients suffering from radiculopathy compared with healthy controls in the NP. For the AF, no significant difference was revealed. In the literature, disc degeneration is considered as one cause of low back pain (LBP) [23]. Additionally, a strong association between disc degeneration and pain has been shown [24] [25]. In accordance with our work, the first degenerative changes could be found in the NP [5]

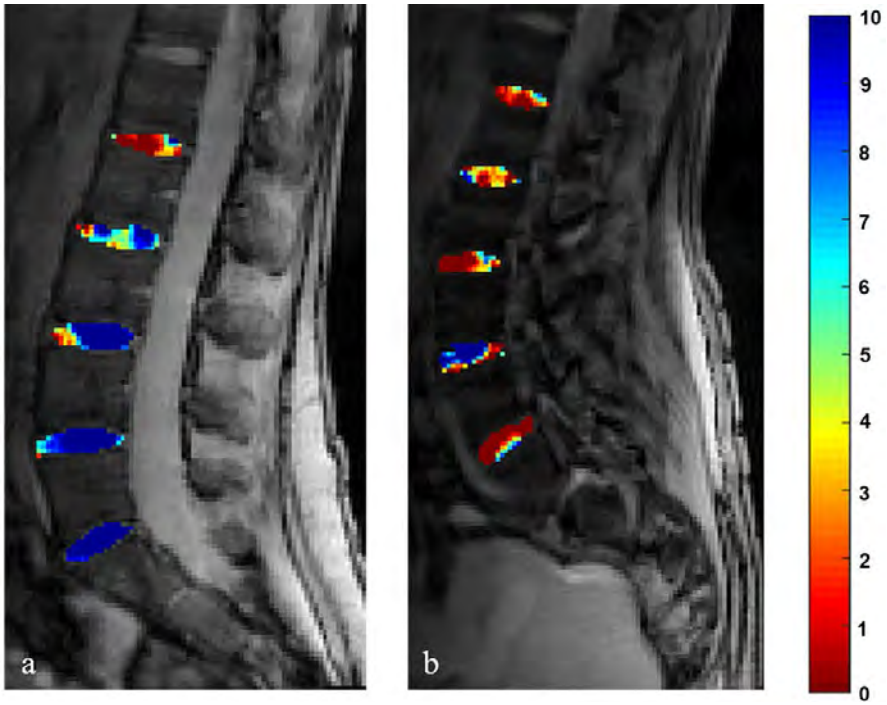

Figure 2. Color-coded gagCEST map with low GAG content in red and high GAG content in blue of the lumbar spine (L1-S1). In this example, a patient suffering from radiculopathy of the segment L5/S1 (image (b)) displayed a significantly lower gagCEST effect compared with a healthy control (image (a)). The low gagCEST effect of segment L1/L2 of the control participant was explainable by motion artifacts of the diaphragm.

[20]. In the AF, degenerative changes are much more difficult to demonstrate due to physiologically lower GAG values in the AF compared with the NP [20]. We found lower GAG values in degenerated IVDs (Pfirrmann grade 3 - 5) compared with healthy discs (Pfirrmann grade 1 and 2) in our data sets. In addition, degenerated discs showed a loss of GAGs in the NP and an adjustment of the AF GAG content in contrast to non-degenerated IVDs, which revealed a significantly higher GAG content of the NP compared with the AF. These findings agree with recent literature demonstrating that our gagCEST sequence works in the context of degenerative IVD changes [5] [17] [20]. According to previous studies, the difference in GAG content between the NP and AF vanished in lumbar IVDs with protrusion or extrusion compared with discs with a normal appearance [16].

Our study has limitations. The main limitation of this study was the small sample size. For morphological (Pfirrmann classification) and biochemical MRI (gagCEST), we did not test intra- and interobserver agreement. The Pfirrmann classification has already demonstrated a good intra- and inter-reader reliability in prior studies [16]. For biochemical MRI, we used an automatic detection of GAG content of the NP and AF. For this reason, we believe that the missing reliability calculation is a minor limitation of both analyses. No gender, age, or BMI differentiation was considered in this study. Müller-Lutz and colleagues showed significantly lower GAG values in IVDs with increasing age and higher

BMI [17]. These findings occur before morphological changes of IVDs could be revealed. This is a major limitation of our study considering the age difference between patients and control group and has to be taken into account in follow up studies. Moreover, for ethical reasons, we could not perform a histological correlation. We believe that the strength of this study is its focus on patients with radiculopathy. The results of our study show promise for evaluation of the effect in a larger population, applied to a patients' pain score or considering the difference between gender and age.

5. Summary

In summary, gagCEST of lumbar IVDs on a clinical 3T MRI system is a powerful, non-invasive tool without use of contrast medium to investigate early disc degeneration, predominantly concerning the NP. Biochemical imaging with gagCEST could provide an early biomarker for GAG loss in IVDs that may be on the way to develop degenerative changes like protrusion or extrusion, bony endplate alterations, formation of osteophytes, and consecutive spinal stenosis. Biochemical imaging of IVDs of patients with radiculopathy revealed that significantly lower GAG values compared with healthy controls, especially in the NP, may indicate an association between pain and IVD degeneration.

Conflicts of Interest

The authors declare that they have no conflict of interest.

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Factors Associated with Social Support Needs of Spouses of Patients with Cancer: Online Survey

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Abstract

Background: A means of assessing the social support needs of spouses of patients with cancer is not available in Japan, yet such individuals are at increased risk of developing psychological difficulties. **Objectives:** The present study aimed (1) to describe the social support needs of spouses of patients with cancer, and (2) to explore factors associated with social support needs of spouses of patients with cancer. **Design:** Spouses (n = 559) of patients with cancer were recruited by registered agents of an online survey company and completed a self-reporting, online questionnaire. **Measurements:** The questionnaires included demographic information and a tool to assess social support needs. **Results:** Factor analysis of social support needs of the spouses of patients with cancer indicated that (1) "social support needs regarding disease and treatment of patient" (54 items) comprised 3 factors ("medical condition and cure", "daily life and social support", "intimacy and employment"), and (2) "social support needs of spouse (19 items)" comprised 2 factors ("family psychological issues and social support" and "intimacy, employment and society"). The ANOVA and T tests showed that "younger age", "under treatment", and "cancer not cured: treatment stopped", "PS1" and "PS 2-4", the presence of "lung cancer", and "recurrence/metastasis" were significant factors (all $p < 0.05$). **Conclusions:** The age of the spouse, treatment status, performance status, site of cancer, and recurrence/metastasis are important factors related to spousal needs for social support. Clinicians should assess these factors and the social support needs of spouses to provide appropriate support.

Keywords

Cancer, Spouse, Social Support Needs, Assessment Tool, Online Survey

1. Introduction

Spouses and partners of cancer patients have an increased risk of psychological difficulties, such as depression, anxiety, impaired self-esteem, somatic complaints, and difficulties experienced within the couple [1] [2] [3] [4]. Psychological distress experienced by spouses continues after the death of the patient. One predictor of higher risk for developing complicated grief is the loss of a spouse [5] [6] [7], and caregivers of patients with cancer have a higher level of depressive symptoms after bereavement if they were the patient's spouse [8]. The assessment of spousal needs is a critical step for determining appropriate support and providing high-quality care to reduce psychological distress between spouses.

Some countries have tools to assess support needs for partners and caregivers of patients with cancer, such as the Supportive Care Needs Survey-Partners and Caregivers (SCNS-P & C) in Australia [9], a comprehensive needs assessment tool for cancer caregivers (CNAT-C) in Korea [10], and the Cancer Survivors' Partners Unmet Needs measure (CaSPUN) [11]. However, a tool for assessing the needs of spouses of patients with cancer is not available in Japan. Nevertheless, it is difficult to use other instruments from other countries after translation, because social support needs reflect factors in the social environment, such as perceptions and emotions related to cancer, healthcare systems, social welfare policies, and work systems. Thus, instead of translating other instruments from other countries, we decided to develop an original tool to assess social support needs for spouses in Japan to provide specific care for individual spousal needs.

One assessment of social issues of Japanese patients with cancer showed that 51.1% of them had experienced some type of social issues within the past five years [12]. Another survey demonstrated that the prevalence of unmet supportive care needs among Japanese cancer survivors was high in medical-psychological and financial domains and relatively low in physical and sexual domains [13]. However, caregiver needs were not highly correlated with patient needs, implying that caregivers have their own needs, and that a separate assessment of caregiver need is needed [10]. Therefore, we aimed to describe social support needs of spouses of patients with cancer based on the Social Problem Checklist (SPC) for Japanese patients with cancer [12] [14] and explore factors associated with social support needs of spouses of patients with cancer.

2. Methods

2.1. Participants

We conducted an online survey of 559 spouses of cancer patients between No-

vember 10th and 29th 2016. The eligibility criteria were as follows: recruited by registered agents of an online survey company (Macromill Inc., Tokyo, Japan), age > 20 years, spouses of patients diagnosed with cancer within the past five years, experienced difficulties regarding disease and treatment of the patient at home, in the workplace, and the neighborhood, able to understand details of this study, able to respond to an online survey, and uninformed about eligibility criteria (to exclude bias before the online survey) (**Figure 1**).

2.2. Procedures

Macromill designed the online questionnaire as "the disease survey". Check boxes and radio buttons were used for each item to answer.

Agents at Macromill were informed about the study purpose and recruited eligible participants online. Agents were paid with points in return for participating in this investigation. They could earn points if they answered all questions, and then they could exchange points for cash, gift certificates, merchandise, or points of business partners.

This study was approved by the ethics committee of the Tokyo Medical and Dental University (M2015-581). The return of completed forms was considered consent. It was explained that participants could stop answering the questionnaire when they did not want to answer.

Participants completed the online self-reporting questionnaires, which included 23 items of spousal demographic data and 146 items regarding social issues and social support needs of spouses of patients with cancer.

2.3. Measured Items

2.3.1. Demographic and Medical Information

The demographic data including sex, age, province, children, annual household income, personal income, spousal occupation, site of cancer, previous treatment, treatment status, recurrence/metastasis, performance status, types of previous and present medical facilities, housemate, and housemate other than spouse.

2.3.2. Tool to Assess Social Support Needs of Spouses of Patients with Cancer (73 Items)

We developed a tool with which to assess social issues of spouses of Japanese patients with cancer (82 items) based on the SPC for Japanese patients with cancer (60 items) [12] [14] because measures of social issues and support needs developed in other countries were not appropriate for Japanese spouses of patients with cancer.

We confirmed the content validity of the original assessment tool of social issues (82 items) in an initial multidisciplinary meeting that included a psychiatrist, a medical social worker, two clinical psychologists, and a certified palliative care nurse in June 2015. Thereafter, we constructed an original scale of social issues and social support needs for the spouses of Japanese patients with cancer (164 items).

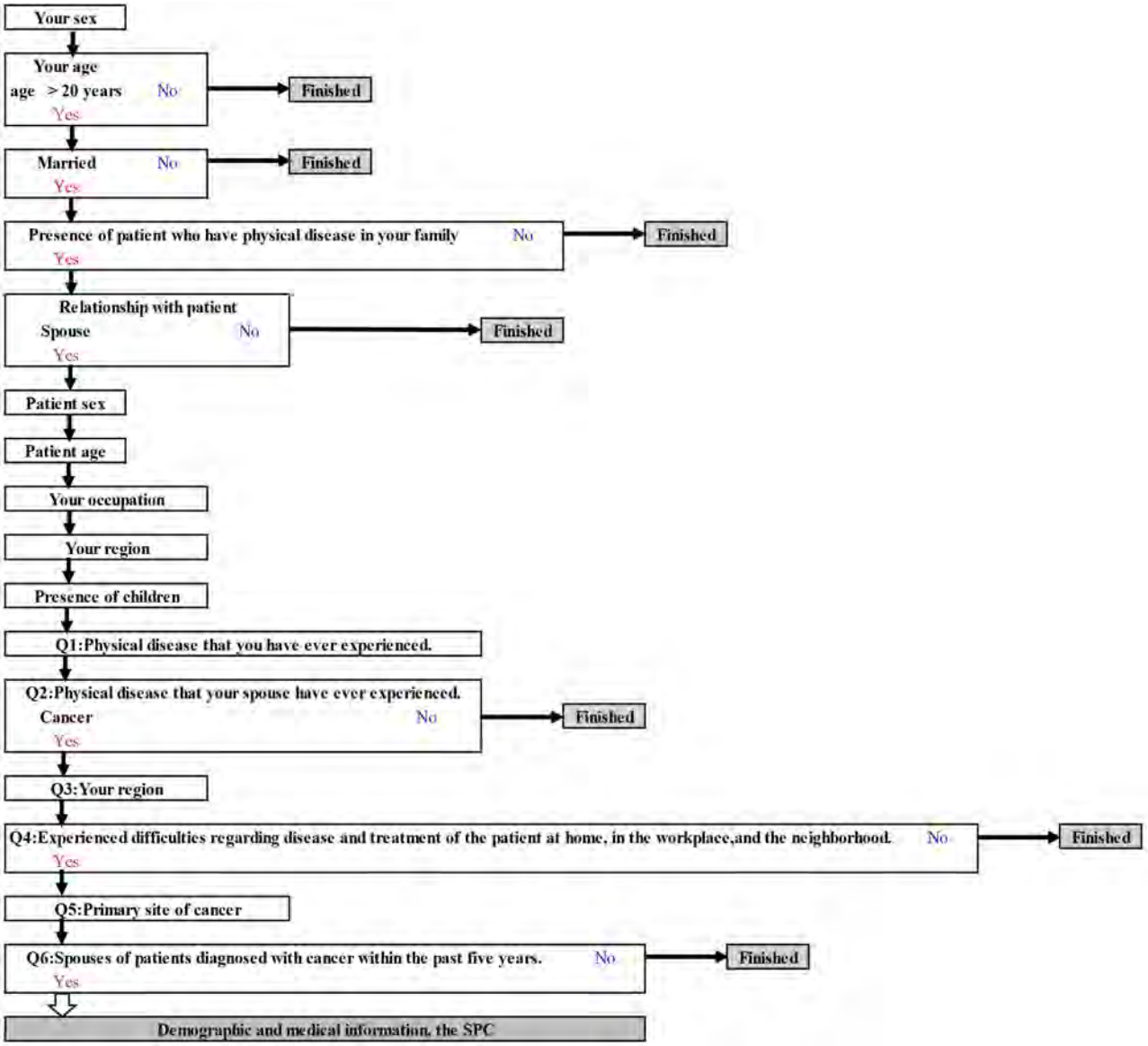

Figure 1. Flow diagram of the process to select participants.

Factor analysis based on Promax rotation using the maximum likelihood method was applied to the scale of social issues and social support needs for the spouses, and 146 items were extracted.

The content validity of this original assessment tool of social issues and social support needs for the spouses of patients with cancer (146 items) was addressed at a second multidisciplinary meeting including a psychiatrist and two clinical psychologists in February 2017. The present article focuses on the 73 social support needs items of these 146 items (**Figure 2**).

The participants were asked if they had needed any supports regarding each item during the past month to rate the level of severity on a 6-point Likert scale, which ranged from 1 (very much) to 2 (quite a lot), 3 (a little), 4 (solved by myself), 5 (extremely satisfied), or 6 (not applicable).

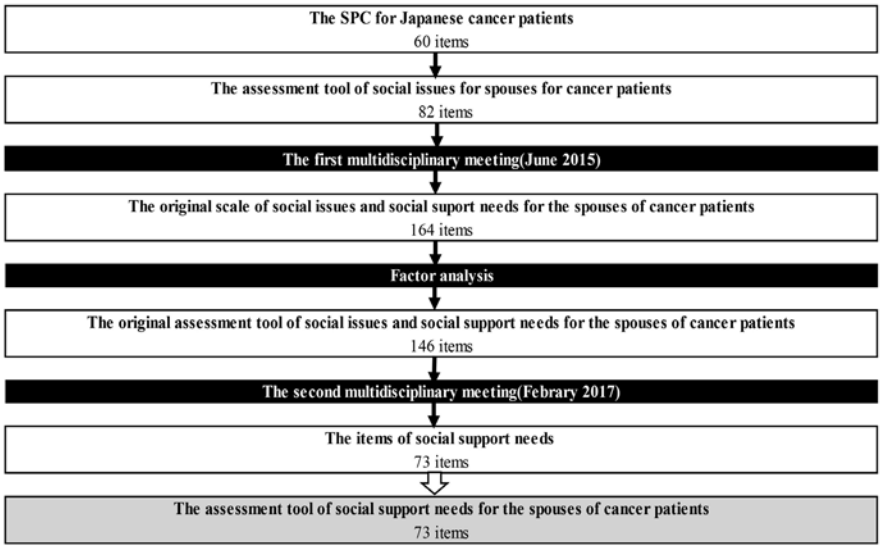

Figure 2. Flow diagram of the process to make the assessment tool.

After a multidisciplinary meeting including a psychiatrist, two clinical psychologists, and a medical social worker to evaluate the assessment tool of social support needs of the spouses of cancer patients, each response category was scored as follows: 4 (very much),3 (quite a lot), 2 (a little), 1 (solved by myself, extremely satisfied), or 0 (not applicable). A rating ≥ 3 was regarded as a serious need for social support.

2.4. Statistical Analysis

The demographic and medical information of the participants is summarized using descriptive statistics.

We modified the 60-item SPC for Japanese patients with cancer [12] [14] and developed an original scale of 164 items regarding social issues and social support needs of their spouses. Factor analysis based on Promax rotation using the maximum likelihood method extracted 146 items regarding social issues and the social support needs of the spouses of patients with cancer.

Differences among three age groups, three performance status groups, and three groups with previous treatment were assessed using ANOVA.

Differences among sex, cancer site, previous treatment, presence of recurrence/metastasis, type of previous and present medical facilities, and housemate other than spouse were evaluated using T-tests. All data were statistically analyzed using SPSS version 23.0 (IBM, Armonk, NY, USA).

3. Results

3.1. Demographic and Medical Information of Participants

We distributed online questionnaires to 699 agents of Macromill and analyzed 559 that were returned (valid response rate, 80.0%). **Table 1** shows the characteristics and medical information of the participants.

Table 1. Demographic and clinical characteristics of participants (n = 559).

Variables	Means ± SD or n (%)
Male spouse	339 (60.6%)
Patient age (y)	55.1 ± 12.5
20s	13 (2.3%)
30s	54 (9.7%)
40s	116 (20.75%)
50s	152 (27.2%)
≥60	224 (40.1%)
Spouse age (y)	54.9 ± 12.7
20s	19 (3.4%)
30s	50 (8.9%)
40s	109 (19.5%)
50s	166 (29.7%)
≥60	215 (38.5%)
Primary site of cancer	
(multiple answers)	
Breast	139 (24.9%)
Colon	95 (17.0%)
Stomach	62 (11.1%)
Lung	59 (10.6%)
Malignant lymphoma	34 (6.1%)
Prostate	28 (5.0%)
Uterine	20 (3.6%)
Liver	18 (3.2%)
Thyroid	18 (3.2%)
Kidney	17 (3.0%)
Esophagus	17 (3.0%)
Bladder	16 (2.9%)
Head, neck, oral	13 (2.3%)
Pancreas	12 (2.1%)
Leukemia	10 (1.8%)
Other	55 (9.8%)
Previous treatment	
Surgery	455 (81.4%)
Radiation	180 (32.2%)
Chemotherapy or hormone therapy	233 (41.7%)
Folk remedy	6 (1.1%)
Other	30 (5.4%)

Continued

Treatment status	
Under treatment	233 (41.7%)
Cancer cured; treatment completed	274 (49.0%)
Cancer not cured; treatment stopped	29 (5.2%)
Other situations	23 (4.1%)
Recurrence/metastasis	
Yes	136 (24.3%)
Performance status	
PS0	322 (57.6%)
PS1	163 (29.2%)
PS2	31 (5.5%)
PS3	20 (3.6%)
PS4	23 (4.1%)
Types of previous and present medical facilities	
(Multiple answers)	
Special hospital for cancer	109 (19.5%)
General hospital	388 (69.4%)
Regional hospital	84 (15.0%)
Local clinic	10 (1.8%)
Other	8 (1.4%)
Unclear	4 (0.7%)
Children	
Yes	100 (17.9%)
Housemate	
Living together	552 (98.7%)
Living apart	7 (1.3%)
Housemate other than spouse	
Yes	327 (58.5%)
Province	
Hokkaido region	27 (4.8%)
Touhoku region	32 (5.7%)
Kantou region	165 (29.5%)
Cyubu region	116 (20.8%)
Kinki region	100 (17.9%)
Cyugoku region	41 (7.3%)
Shikoku region	13 (2.3%)
Kyusyu region	65 (11.6%)

Continued

Annual household income	
Under 2 million	17 (3.0%)
2 - under 4 million	112 (20.0%)
4 - under 6 million	156（27.9%)
6 - under 8 million	100 (17.9%)
8 - under 10 million	54 (9.7%)
10 - under 12 million	21 (3.8%)
12 - under 15 million	17 (3.0%)
15 - under 20 million	17 (3.0%)
20 million and over	9 (1.6%)
Unclear	29 (5.2%)
Non-response	27 (4.8%)
Personal income	
Under 2 million	188 (33.6%)
2 - under 4 million	122 (21.8%)
4 - under 6 million	87 (15.6%)
6 - under 8 million	57 (10.2%)
8 - under 10 million	25 (4.5%)
10 - under 12 million	15 (2.7%)
12 - under 15 million	7 (1.3%)
15 - under 20 million	3 (0.5%)
20 million and over	2 (0.4%)
Unclear	23 (4.1%)
Non-response	30 (5.4%)
Spousal occupation	
Civil servant	16 (2.9%)
Manager & Executive	18 (3.2%)
Office worker (Cletical work)	70 (12.5%)
Office worker (Technical work)	65 (11.6%)
Office worker (Other)	62 (11.1%)
Indipendent business	42 (7.5%)
Liberal profession	8 (1.4%)
Homemaker	118 (21.1%)
Part time job	60 (10.7%)
Student	1 (0.2%)
Other	27 (4.8%)
Unemployed	72 (12.9%)

The spouses were divided into young (age 20s and 30s; n = 69, 12.3%), middle-aged (age 40s and 50s; n = 275, 49.2%), and elderly (age ≥ 60s; n = 215, 38.5%) groups. The primary sites of cancer comprised breast (n = 139, 24.9%), colon (n = 95, 17.0%), stomach (n = 62; 11.1%), lung (n = 59, 10.6%), and malignant lymphoma (n = 34, 6.1%). The types of therapy were surgery (n = 455, 81.4%) and chemotherapy or hormonal therapy (n = 233, 41.7%). The cancer had been cured and treatment had been completed in 274 (49.0%) patients, and 233 (41.7%) were still undergoing treatment.

3.2. Factors Related to Social Support Needs

3.2.1. Factors Related to Social Support Needs Regarding Disease and Treatment of Patients

Factor analysis identified three factors among 54 items (Table 2).

Former items 1, 24, 36, 46 and 47 did not load < 0.1 about the differences of factor loading between factors and were excluded (**Appendix A**).

A psychiatrist and a clinical psychologist at a multidisciplinary meeting discussed the items that were included in different factors between the assessment tools of social issues and of social support needs and excluded former item 59 (the number before exclusion) that did not differ highly in factor loading between factors.

3.2.2. Factors Related to Social Support Needs of Spouses

Factor analysis identified two factors among 19 items (Table 3).

Former item 22 did not load < 0.1 about the differences of factor loading between factors and was excluded (**Appendix B**).

A psychiatrist and a clinical psychologist at a multidisciplinary meeting discussed the items that were included in different factors between the assessment tools of social issues and of social support needs and excluded former items 16 and 18 (numbers before exclusion) that did not significantly differ between factors.

3.3. Factors Associated with Social Support Needs of Spouses of Patients with Cancer

Table 4 shows that scores were higher across all scales and factors of social support needs in younger than in middle-aged and elderly spouses ($p < 0.05$).

Among the most prevalent of the primary cancer sites, namely lung cancer (10.6%), stomach cancer (11.1%), colon cancer (17.0%) and breast cancer (24.9%), only lung cancer significantly differed in terms of social support needs regarding "patient disease and treatment" and "spouse difficulties" (both $p < 0.05$).

Table 5 shows that Factor 1, "medical condition and cure" and 2, "daily life and social support" were significantly higher ($p < 0.05$ for both) in the measure of "patient disease and treatment" for patients with than without lung cancer. Factor 1, "family psychological issues and social support", was also significantly

Table 2. Factors of social support needs regarding disease and treatment of patients.

Q: Due to patient's disease and treatment, have you ever needed some support (e.g., advice, information and services from professionals and patients under similar circumstances) during the past month regarding the following items?

	Items	Factor 1	Factor 2	Factor 3
		Medical condition and Cure	Daily life and Social Support	Intimacy and Employment
1	Insufficient information regarding how to take care of patient from now on	0.938	−0.007	−0.034
2	Insufficient information about the methods, contents, and results of medical examinations for patient	0.935	0.009	−0.044
3	Insufficient information regarding patient's current and prospective medical conditions	0.933	−0.021	−0.002
4	Insufficient explanation from medical staff regarding treatment plans and policies	0.909	−0.055	0.072
5	Insufficient information about patient's various treatment plan	0.890	0.030	−0.021
6	Insufficient information regarding how to cope with patient's treatment side effects and disease symptoms	0.865	0.028	0.011
7	Insufficient explanation concerning the benefits and side effects of treatments from medical staff before you decide to have them	0.858	0.107	−0.059
8	Insufficient information regarding patient's appropriate nutrition and diet	0.856	0.016	0.031
9	Insufficient information regarding how to obtain informations about patient's disease and treatment	0.844	0.087	−0.024
10	Choosing which hospital (or doctor) patient should visit to receive medical treatment and examination	0.823	0.093	−0.037
11	Insufficient information concerning how to cope with patient's anxiety and depressive mood	0.773	0.159	−0.025
12	Patient's difficulties in openly communicating with his/her doctor	0.758	0.108	0.057
13	Poor communication among health care staff to coordinate patient's medical treatment and care(e.g., doctor in charge, other doctor, family doctor, and nurse)	0.746	−0.008	0.165
14	Insufficient information regarding patient's palliative medicine and care that decrease distress, such as pain	0.745	0.039	0.122
15	Not being assured that patient's current hospital (or patient's home doctor) would provide advice to him/her when patient have sudden physical problems at home	0.745	0.124	−0.013
16	Having patient's physical problems doesn't treated immediately by medical staff (e.g., doctor, nurse)	0.714	0.105	0.076
17	Little sympathy and support concerning patient's psychological issues from medical staff	0.708	−0.013	0.189
18	Consulting with another specialist besides patient's doctor about his/her disease and treatment	0.650	0.233	−0.080
19	Having no particular clinic and doctor patient or family can consult with when patient and family need to	0.649	0.239	−0.018
20	Insufficient information regarding patients' complementary and alternative medicine (e.g., health foods, hot spring, and qigong)	0.628	0.121	0.117
21	Having access to professional psychological counseling whenever patient and you need it	0.507	0.317	0.015
22	Getting to, entering, leaving, or changing a patient's hospital	0.473	0.292	0.061
23	Imposing a patient's burden on his/her family	−0.073	0.902	0.059
24	Difficulties in patient's dealing with anxiety and worry among his/her family members	0.099	0.859	−0.035

Continued

25	Difficulties enjoying patient's hobbies, recreations and social activities as before because of his/her disease and treatment	0.093	0.844	−0.042
26	Managing patient's and your properties	−0.038	0.834	0.041
27	Patient's difficulties performing his/her responsibilities in the house	0.028	0.828	0.045
28	Medical and living expenses while receiving treatment	0.034	0.822	−0.073
29	Difficulties in having sufficient support available to you (e.g., assistance and public service from people around you)	0.120	0.808	−0.054
30	Planning patient's own and your future life	0.103	0.786	−0.025
31	Taking care of family (e.g., childrearing, nursing parents and spouse)	0.099	0.765	0.009
32	Insufficient information concerning patient's social welfare services (e.g., nursing care insurance program, welfare system for the disabled)	0.182	0.744	−0.064
33	Patient's having no one with a similar experience to talk to	0.230	0.643	0.051
34	Relationship and communication with patient's friends and persons close to patient	0.144	0.642	0.146
35	Changes of patient's figure and appearance(e.g., increasing and decreasing of weight)	0.114	0.630	0.117
36	Relationship and communication with patient's neighbors	0.102	0.618	0.197
37	Using financial services(e.g., loans, health care and life insurance)	0.051	0.608	0.160
38	Having no one and place to go for advice regarding patient's disease and medical treatment life	0.298	0.608	0.016
39	Relationship and communication with your spouse	0.156	0.599	0.143
40	Talking about patients disease with people in the patient's workplace and other social places	0.107	0.571	0.258
41	Patient's being socially isolated	0.231	0.559	0.123
42	Insufficient information concerning living supports for patient receiving cancer \treatment (e.g., medical wig, elastic stockings ,wheelchair, adjustable medical bed)	0.196	0.516	0.176
43	Difficulties in having enough understanding and cooperation from patient's family regarding patient's disease and treatment	0.255	0.505	0.182
44	Talking about patient's disease with patient	0.237	0.502	0.179
45	Being excessively concerned about patient by you	0.151	0.499	0.260
46	Patient's taking care of domestic chores (e.g. cleaning, watching, cooking, grocery shopping)	0.084	0.492	0.333
47	Patient's looking after himself/herself (e.g., eating, bathing, excreting, dressing)	0.307	0.477	0.104
48	Being demoted and assigned a lower position in the patient's workplace	−0.010	−0.033	0.996
49	Difficulties in getting a promotion and advancing patient's career	−0.005	−0.046	0.990
50	Being urged to resign and lose patient's job	−0.003	0.018	0.906
51	Difficulty with asking for time off from patient's work for medical treatment (or school if patient is a student)	0.029	0.135	0.781
52	Issues with patient's pregnancy and childbirth	0.063	0.054	0.732
53	Difficulty with returning and maintaining to patient's work (or education if he/she is a student)	0.005	0.321	0.583
54	Matters concerning your sexual life	0.023	0.250	0.562
	Factor 1			
Inter-factor correlation	**Factor 2**	0.898**		
	Factor 3	0.733**	0.814**	

Factor extraction method: maximum likelihood method; Factor rotation method: Promax; Number of rotation: 4; Factor loadings > 0.25; **p < 0.01.

Table 3. Factors of social support needs for spouses.

Q: Have you ever needed support for yourself (e.g., advice, information and services from professionals and patients under similar circumstances) during past month regarding the following items?			
	Items	**Factor1**	**Factor2**
		Family's psychological issue and Social support	**Intimacy, Employment and Society**
1	Difficulties in dealing with your anxiety and worry as family	1.044	−0.175
2	Having no one and place to go for advice for you regarding patient's disease and medical treatment life	0.859	0.031
3	Feeling burden as family	0.855	0.051
4	Insufficient information concerning how to cope with your anxiety and depressive mood as family	0.849	−0.051
5	Having no one with a similar experience to talk to	0.829	0.072
6	Difficulties performing your responsibilities in the house	0.792	0.108
7	Difficulties enjoying your hobbies, recreations and social activities as before because of patient disease and treatment	0.762	0.108
8	Taking care of domestic chores (e.g., cleaning, washing, cooking, grocery shopping)	0.750	0.080
9	Being socially isolated	0.738	0.168
10	Relationship and communication with your friends and persons close to you	0.666	0.259
11	Little sympathy and support concerning your psychological issues from medical staff	0.642	0.157
12	Talking about patient's disease in your work place and other social places	0.592	0.340
13	Relationship and communication with your neighbors	0.553	0.394
14	Being excessively concerned about you by patient	0.533	0.375
15	Being demoted and assigned a lower position in your workplace because of patients disease	−0.068	1.010
16	Difficulties in getting a promotion and advancing in your workplace because of patient's disease	−0.086	0.991
17	Being urged to resign and lose your work	−0.058	0.951
18	Issues with your pregnancy and childbirth	0.063	0.753
19	Facing discriminatory treatment because of patient's disease	0.291	0.666
Inter-factor correlation	**Factor 1**		
	Factor 2	0.760**	

Factor extraction method: maximum likelihood method; Factor rotation method: Promax; Number of rotation: 3; Factor loadings > 0.25; **p < 0.01.

higher ($p < 0.05$) in the measure of "spouse difficulties" for patients with than without lung cancer.

All scales and factors of social support needs were significantly higher for "under treatment" than "cancer cured: treatment completed" ($p < 0.05$) and for "cancer not cured: treatment stopped" than "cancer cured: treatment completed" ($p < 0.05$).

Table 6 shows that scores for all factors regarding "patient disease and treatment" were significantly higher for patients with than without recurrence/metastasis ($p < 0.05$) and "spouse difficulties" ($p < 0.05$).

Table 4. Comparisons of mean age and social support needs (ANOVA).

				Mean(SD)			F value	Multiple Comparison
				Young (N = 69)	Middle (N = 275)	Elderly (N = 215)		
Social Support Needs	Patient disease and treatment	Total		85.1 (70.7)	51.2 (52.2)	48.1 (40.4)	14.8*	Y > M, Y > E
		Factor 1	Medical condition and cure	36.2 (30.2)	23.1 (23.5)	23.1 (19.7)	9.7*	Y > M, Y > E
		Factor 2	Daily life and social support	38.5 (32.4)	23.0 (24.4)	21.7 (19.0)	14.1*	Y > M, Y > E
		Factor 3	Intimacy and employment	10.4 (9.6)	5.0 (6.4)	3.3 (5.1)	31.2*	Y > M, Y > E, M > E
	Spouse difficulties	Total		30.7 (25.1)	14.8 (16.9)	13.1 (13.3)	30.1*	Y > M, Y > E
		Factor 1	Family psychological issues and social support	23.2 (18.5)	12.1 (13.2)	11.2 (10.8)	23.5*	Y > M, Y > E
		Factor 2	Intimacy, employment and society	7.5 (6.9)	2.7 (4.4)	1.8 (3.3)	44.5*	Y > M, Y > E

*p < 0.05. Y, young; M, middle-aged; E, elderly. Ages: Y, M and E: 21 - 39, 40 - 59, 60 - 87 years, respectively.

Table 5. Comparisons of mean cancer sites and social support needs (T test).

				M(SD)		t ratio
				Lung Cancer		
				Presence (N = 59)	Absence (N = 500)	
Social Support Needs	Patient disease and treatment	Total		68.6 (53.8)	52.4 (51.7)	2.3*
		Factor 1	Medical condition and cure	30.6 (24.6)	24.0 (23.2)	2.0*
		Factor 2	Daily life and social support	31.5 (25.2)	23.6 (24.0)	2.4*
		Factor 3	Intimacy and employment	6.5 (7.9)	4.8 (6.7)	1.8
	Spouse difficulties	Total		21.5 (18.4)	15.5 (17.6)	2.5*
		Factor 1	Family psychological issues and social support	17.5 (14.1)	12.6 (13.5)	2.6*
		Factor 2	Intimacy, employment and society	4.0 (5.1)	2.8 (4.7)	1.8

*p < 0.05.

Table 6. Comparisons of mean recurrence/metastasis and social support needs (T test).

				M(SD)		t ratio
				Recurrence/metastasis		
				Presence (N = 136)	Absence (N = 423)	
Social Support Needs	Patient disease and treatment	Total		77.0 (57.1)	46.8 (48.2)	5.56*
		Factor 1	Medical condition and cure	35.3 (26.0)	21.3 (21.5)	5.68*
		Factor 2	Daily life and social support	34.7 (26.3)	21.1 (22.6)	5.39*
		Factor 3	Intimacy and employment	7.0 (8.4)	4.4 (6.1)	3.41*
	Spouse difficulties	Total		22.9 (20.2)	13.9 (16.3)	4.7*
		Factor 1	Family psychological issues and social support	18.6 (15.2)	11.4 (12.7)	5.0*
		Factor 2	Intimacy, employment and society	4.3 (5.8)	2.5 (4.3)	3.4*

*p < 0.05

"PS1" and "PS 2 - 4" scored higher in all scales and factors of social support needs than "PS0" ($p < 0.05$ for both).

4. Conclusions

The present study described the social support needs of spouses of patients with cancer. This allowed the first systematic and comprehensive needs assessment of these individuals in Japan. Factor analysis identified three underlying domains in "social support needs regarding patient disease and treatment" ("medical condition and cure", "daily life and social support", and "intimacy and employment") and two underlying domains in "social support needs regarding spouse difficulties" ("family psychological issues and social support" and "intimacy, employment and society"). These domains reflect the common needs generally reported in the literature regarding cancer caregivers [10] [11] [15]. One advantage of the present study is that the tool for assessing social support needs focused on the spouses of patients with cancer and identified needs for support with "patient disease and treatment" and "spouse difficulties".

The ANOVA findings showed that the young group scored significantly higher across all scales and factors of social support needs than the middle-aged and elderly groups. In the Adolescent and Young Adult (AYA) population, cancer is the leading cause of death due to illness with the lowest mortality rate, as well as in children, and AYA patients with cancer are growing independently, starting life within the community, and are reaching reproductive age [16]; they have physical changes to become adults and unique psychological issues that are distinct from those of pediatric and older adult patients [16]. Intimacy is harmed more frequently among survivors of breast cancer aged < 45 years than among those aged 46 - 54 and > 55 years. Psychological problems are more prevalent among younger women who survive breast cancer [17]. AYA cancer patients experienced many specific sequelae after cancer diagnosis and treatment, such as loss of fertility [18] [19], hair loss, and other physical changes and fatigue [20] [21], as well as difficulties with social relationships, employment, educational attainment, and financial burden [22] [23]. Regarding mental health, AYA cancer patients had an increased prevalence of anxiety, depression, and distress than healthy peers and the general public [24] [25]. Additionally, AYA cancer survivors were more likely to have a poorer quality of life than persons of the same age in the general population and older cancer survivors [26]. In young patients, these subjective factors of severity of illness and emotional distress affected post-traumatic stress symptoms and subjective experience of her spouse more [27] [28]. In particular, younger age is a factor associated with high psychological distress and a low quality of life for spouses and partners [29]. The present findings were consistent with those of a previous study of AYA cancer caregivers and indicated that medical staff should consider the social support needs of young spouses more carefully.

Only lung cancer significantly differed among primary cancer sites in patients.

According to the website of the Cancer Information Service of National Cancer Center in Japan [30], lung cancer had the highest number of fatalities among males and females during 2017. Lung cancer is associated with increased symptomatic distress and unmet needs compared with other types of cancer, with the most common symptoms being fatigue, cough, and dyspnea [31]. Therefore, lung cancer also impacts anxiety and depression among spouses [32] [33]. This result is consistent with previous finding that the most prevalent unmet needs among caregivers of patients with advanced lung cancer were related to information, healthcare service, and daily living [34], and that unmet needs were more prevalent among caregivers of lung cancer survivors at 6 and 24 months after diagnosis [15]. The present study did not find significant differences regarding "intimacy and employment" in disease and treatment of patient and "intimacy, employment and society" in spousal difficulties. Being a young spousal caregiver is a factor of economic burden for the spouse of a patient with lung cancer [35]. In this study, most participants were more than middle-aged, and, therefore, issues of intimacy and employment might have been less important.

It was found that the social support needs were significantly higher among spouses of patients in the "under treatment" than in the "cancer cured: treatment completed" groups and among those of patients in "cancer not cured: treatment stopped" than in "cancer cured: treatment completed" groups. Patients undergoing surgery, chemotherapy, radiation therapy, or chemoradiotherapy experience significant cancer treatment-related fatigue that begins during treatment and decreases following treatment [36], and patients' fatigue might also affect spousal distress and social support needs. For example, the spouses of patients with incurable cancer in the palliative care phase have a higher frequency of depression symptoms [37], and spouses faced with the "cancer not cured: treatment stopped" situation also have many social support needs. Our results are consistent with previous findings and indicate that the spouses of patients with cancer have obvious social support needs, especially when patients undergo treatment or are incurable and treatment has been stopped. Appropriate social support should be provided in such situations.

We also found higher social support needs among spouses of patients with than without recurrence or metastasis, with PS1 than PS0, and with PS 2 to 4 than PS0. Patients who have cancer with poorer performance status might have difficulty coping with the disease and consequently might experience more psychological distress [38]. Therefore, their spouses might also have high levels of need when their patients have low performance status. Indeed, recent studies have suggested that spouses and partners experience physical and psychological distress over the burden of patient care, anxiety, depression, and posttraumatic stress symptoms, and they have to deal with their own lifestyles (such as childcare, missing work, financial burden) when patients are in the terminal phase or developing metastasis and recurrence [37] [39] [40] [41] [42]. Furthermore, caregiver depression, burden, and missing work increase more during the terminal period than during the palliative period [40]. This finding suggests that medical

staff should assess the social support needs of spouses in these situations to provide adequate support as the functional status of the patient declines.

In conclusion, the age of the spouse, cancer site in the patient, recurrence/metastasis, and performance status are important factors related to the social support needs of spouses. Medical staff should assess the situations of spouses on an individual basis and plan strategies to help reduce unmet needs. Communicating openly with spouses and consulting with health-care professionals could be helpful to fulfill specific social support needs regarding disease and treatment of patients and specific ones of spouses. Providing concrete medical information would be effective to solve needs of disease and treatment of patients. Introducing psychosocial services and self-help groups would be also useful to solve being socially isolated and having no one with a similar experience to talk to. Delivering psychoeducation on relaxation techniques and communication skills with patient, medical staff, people in workplace may improve spouse coping skills to solve psychosocial needs by themselves [43] [44]. Couple-based interventions may also beneficial for spouses to improve relational satisfaction and communication with patients [45] [46].

The most important study limitation is the representativeness of the sample. The participants might have been affected by sampling bias because they were recruited by agents online and, therefore, targeted only persons who were familiar with the internet and specific agents. An online survey that does not reflect whether everything recorded about the patients was correct was used. Distribution of cancer sites was slightly different from that of the general cancer population in Japan. Social support needs might have been underestimated because most participants had good performance status. The second limitation is that our original tool has not been standardized, although its content validity was confirmed in a multidisciplinary meeting.

Despite the limitations, this is the first study to examine the social support needs and their associated factors among spouses of patients with cancer in Japan.

In future research, clinical data about spouses of cancer patients in the hospital setting should be collected. We therefore plan to test the applicability of the assessment tool in hospitals and to explore the social support needs for spouses of patients with cancer in Japan.

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Conflicts of Interest

The authors have no conflicts of interest to declare.

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Appendix A. The original 60-item of social support needs regarding disease and treatment of patients.

	Q: Due to patient's disease and treatment, have you ever needed support (such as advice from professionals and patients under similar circumstances, information and services) during the past month regarding the following items?	
	1) Very much 2) Quite a lot 3) A little 4) Solved by myself 5) Extremely satisfied 6) Not applicable	
1	About outpatient visit.	1 2 3 4 5 6
2	**Going to, entering, leaving, or changing a patient's hospital.**	1 2 3 4 5 6
3	**Consultation with another specialist besides patient's current doctor about disease and treatment.**	1 2 3 4 5 6
4	**Having access to professional psychological counseling whenever patient and you needed.**	1 2 3 4 5 6
5	**Having physical problems of patient not immediately treated to by medical staff (e.g., doctor, nurse).**	1 2 3 4 5 6
6	**Little sympathy and support concering patient's psychological issues from medical staff.**	1 2 3 4 5 6
7	**Poor communication among health care staff to coordinate medical treatment and care of patient (e.g., doctor in charge, other doctors, family doctor and nurses).**	1 2 3 4 5 6
8	**Not being assured that current hospital or home doctor would provide advice when patients have sudden physical problems at home.**	1 2 3 4 5 6
9	**Having no particular clinic and doctor that patient or family could consult when patient and family feel the need.**	1 2 3 4 5 6
10	**Choosing which hospital (or doctor) patient should visit to receive medical treatment and examinations.**	1 2 3 4 5 6
11	**Insufficient information about methods, content, and results of medical examinations of patient.**	1 2 3 4 5 6
12	**Insufficient information regarding how to obtain information about disease and treatment of patient.**	1 2 3 4 5 6
13	**Insufficient information about various treatment plans for patient.**	1 2 3 4 5 6
14	**Insufficient explanations concering benefits and side effects of treatments from medical staff before you decide to have them,.**	1 2 3 4 5 6
15	**Insufficient information regarding complementary and alternative medicine (e.g., health foods, hot springs and qigong etc).**	1 2 3 4 5 6
16	**Insufficient information regarding palliative medicine and care to decrease distress of patient, such as pain.**	1 2 3 4 5 6
17	**Insufficient explanation from medical staff about treatment plans and policies.**	1 2 3 4 5 6
18	**Insufficient information regarding current and prospective medical condition of patient.**	1 2 3 4 5 6
19	**Insufficient information regarding how to cope with side effects of treatments and disease symptoms.**	1 2 3 4 5 6
20	**Insufficient information regarding how to take care of patient from now on.**	1 2 3 4 5 6
21	**Insufficient information regarding appropriate nutrition and diet for patient.**	1 2 3 4 5 6
22	**Insufficient information regarding how to cope with patient's anxiety and depressive mood.**	1 2 3 4 5 6
23	**Difficulties for patient in openly communicating with doctor.**	1 2 3 4 5 6
24	Getting around and moving of patient (including transportation).	1 2 3 4 5 6
25	**Patient looking after self (e.g., eating, bathing, excreting, dressing).**	1 2 3 4 5 6
26	**Patient taking care of domestic chores for patient (e.g., cleaning, washing, cooking, grocery shopping).**	1 2 3 4 5 6
27	**Difficulties in having enough understanding and cooperation from patient's family regarding disease and treatment of patient.**	1 2 3 4 5 6

Continued

28	**Taking care of family (e.g., childrearing, nursing parents and spouse).**	1 2 3 4 5 6
29	**Imposing a burden on family imposed by patient.**	1 2 3 4 5 6
30	**Patient having difficulty dealing with anxiety and worry among family members.**	1 2 3 4 5 6
31	**Difficulties in having sufficient support available to you (e.g., assistance and public service from people around you).**	1 2 3 4 5 6
32	**Difficulties for patient performing responsibilities in the house.**	1 2 3 4 5 6
33	**Planning patient's and your own future.**	1 2 3 4 5 6
34	**Issues with pregnancy and childbirth for patient.**	1 2 3 4 5 6
35	**Being excessively concerned about patient by you.**	1 2 3 4 5 6
36	Diffferenecs in opinions about disease and treatment of patient between you and patient.	1 2 3 4 5 6
37	**Talking about patient's disease with patient.**	1 2 3 4 5 6
38	**Matters concerning your sex life.**	1 2 3 4 5 6
39	**Relationship and communication with spouse.**	1 2 3 4 5 6
40	**Relationships and communication with patient's friends and others close to patient.**	1 2 3 4 5 6
41	**Relationships and communication with patient's neighbors.**	1 2 3 4 5 6
42	**Talking about patient's disease with people in the patient's workplace and other social places.**	1 2 3 4 5 6
43	**Patient having no-one with similar experience to talk to.**	1 2 3 4 5 6
44	**Patient being socially isolated.**	1 2 3 4 5 6
45	**Having no one or place to go for advice regarding patient's disease and medical treatment.**	1 2 3 4 5 6
46	Patient facing discriminatory treatment because of patient's disease.	1 2 3 4 5 6
47	Changes in other people's attitudes and behaviors toward patient.	1 2 3 4 5 6
48	**Difficulties enjoying hobbies, recreations and social activities for patient as before because of disease and treatment.**	1 2 3 4 5 6
49	**Changes in appearance of patient (e.g., increased or decreased weight).**	1 2 3 4 5 6
50	**Difficulties for patient to return to and maintain to work (or education if patient is a student).**	1 2 3 4 5 6
51	**Difficulties for patient to ask for time off from work (or school if student) for medical treatment.**	1 2 3 4 5 6
52	**Difficulties for patient in getting a promotion and advancing career.**	1 2 3 4 5 6
53	**Patient being demoted and assigned a lower position in workplace.**	1 2 3 4 5 6
54	**Patient being urged to resign and lose job.**	1 2 3 4 5 6
55	**Medical and living expenses while receiving treatment.**	1 2 3 4 5 6
56	**Using financial services (e.g., loans, health care and life insurance).**	1 2 3 4 5 6
57	**Managing patient's and your properties.**	1 2 3 4 5 6
58	**Insufficient information concering social welfare services (e.g., nursing care insurance program, welfare system for the disabled).**	1 2 3 4 5 6
59	Insufficient information concerning community health care services availrable to patient (e.g., home visit by a doctor and nurse)	1 2 3 4 5 6
60	**Insufficient information concerning living supports for patient receiving cancer treatment (e.g., wigs, elastic stockings, wheelchair, adjustable medical bed).**	1 2 3 4 5 6

Items in bold letters were selected and modified for the original 54-item social support needs regarding disease and treatment of patient used in this study.

Appendix B. The original 22-item of social support needs of spouses.

Q: Have you ever needed support for yourself (such as advice from professionals, similar patients, information and services) during the past month regarding the following items?							
1) Very much 2) Quite a lot 3) A little 4) Solved by myself 5) Extremely satisfied 6) Not applicable							
1	**Little sympathy and support concerning your psychological issues from medical staff.**	1	2	3	4	5	6
2	**Insufficient information concerning how to cope with your anxiety and depressive mood as a family.**	1	2	3	4	5	6
3	**Taking care of domestic chores (e.g., cleaning, washing, cooking, grocery shopping).**	1	2	3	4	5	6
4	**Feeling burdened as a family.**	1	2	3	4	5	6
5	**Difficulties dealing with your anxiety and worry as family.**	1	2	3	4	5	6
6	**Difficulties performing your responsibilities in the house.**	1	2	3	4	5	6
7	**Issues with your pregnancy and childbirth.**	1	2	3	4	5	6
8	**Being excessively concerned about you by patient.**	1	2	3	4	5	6
9	**Relationships and communication with your friends and persons close to you.**	1	2	3	4	5	6
10	**Relationships and communication with neighbors.**	1	2	3	4	5	6
11	**Talking about patient's disease in your workplace and other social places.**	1	2	3	4	5	6
12	**Having no one with similar experience to talk to.**	1	2	3	4	5	6
13	**Being socially isolated.**	1	2	3	4	5	6
14	**Having no one or place to go for advice regarding patient's disease and medical treatment life.**	1	2	3	4	5	6
15	**Facing discriminatory treatment because of patient's disease.**	1	2	3	4	5	6
16	Changes in other people's attitudes and behaviors toward you because of patient's disease.	1	2	3	4	5	6
17	**Difficulties enjoying your hobbies, recreations and social activities as before because of disease and treatment of patient.**	1	2	3	4	5	6
18	Difficulties for you to return to and maintain to work (or education if patient is a student) because of patient's disease.	1	2	3	4	5	6
19	**Difficulties in getting a promotion and advancing in your workplace because of patient's disease.**	1	2	3	4	5	6
20	**Being demoted and assigned a lower position in your workplace because of patient's disease.**	1	2	3	4	5	6
21	**Being urged to resign and lose your job.**	1	2	3	4	5	6
22	Insufficient information concering social welfare services (e.g., family care leave, family care leave benefits).	1	2	3	4	5	6

Items in bold letters were selected and modified for the original 54-item social support needs regarding disease and treatment of patient used in this study.

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Discussion on the Effectiveness of Elevating HDL-C in Treating Cardiovascular Diseases of Patients with Type 2 Diabetes Mellitus

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Abstract

High density lipoprotein protects cardiovascular diseases and reduces the risk of cardiovascular diseases through cholesterol reverse transport and other mechanisms. High-density lipoprotein cholesterol (HDL-C) is an independent predictor of negative events in cardiovascular diseases. Low concentration of HDL-C indicates abnormal regulation of HDL anabolism. Various proteins and receptors such as cholesteryl ester transfer protein (CETP) are involved in HDL anabolism. Type 2 Diabetes Mullitu and its related metabolic syndrome, chronic inflammation as well as oxidative stress not only affect the proteins and receptors related to HDL anabolism, but also affect their functional changes, making HDL change from anti-inflammatory, antioxidant, protecting endothelial cell function to pro-inflammatory, pro-oxidative and pro-endothelial cell apoptosis. This article will describe the relationship between HDL-C, type 2 diabetes and cardiovascular diseases from the effects of T2DM on HDL anabolism and function, and further explore the effectiveness of elevating HDL-C in treating cardiovascular diseases of patients with type 2 diabetes.

Keywords

HDL, Type 2 Diabetes Mellitus, Cardiovascular Diseases, Reverse Cholesterol Transport

1. Introduction

Patients with diabetes mellitus are high-risk groups for cardiovascular and cerebrovascular diseases. The risk of cardiovascular and cerebrovascular diseases in

patients with diabetes mellitus is 2 - 4 times higher than that of non-patients with diabetes mellitus. Compared with non-patients with diabetes mellitus with coronary heart disease, patients with diabetes mellitus with coronary heart disease account for 67% of coronary heart disease-related deaths. About 84% of patients with diabetes mellitus aged over 65 die from cardiovascular and cerebrovascular events [1]. Therefore, diabetes patients, a large group of people, have attracted extensive attention. Both type 2 diabetes mellitus and its metabolic syndrome can affect HDL [2]. Moreover, chronic inflammation, oxidative stress and other factors of diabetes can lead to changes in HDL serum concentration, structure and function, and even change from functional HDL with anti-inflammation, anti-oxidation, anti-apoptosis and anti-thrombosis to dysfunction HDL with pro-inflammation, pro-oxidation, pro-apoptosis and pro-thrombosis functions, thus increasing the risk of cardiovascular diseases. As a result, the effectiveness of elevating HDL-C as a therapeutic target to reduce the risk of cardiovascular diseases in patients with type 2 diabetes mellitus has been widely questioned. This article will review the effectiveness of elevating HDL-C in treating cardiovascular diseases in patients with type 2 diabetes mellitus from the aspects of the effects of type 2 diabetes mellitus on HDL anabolism and function.

2. HDL

2.1. Structure

In plasma, HDL is the lipoprotein with the largest density and the smallest volume in human body (Density: 1.063 - 1.21 g/ml) [3]. The main protein component of HDL is apolipoprotein A-I(apo A-I), accounting for about 70%. Therefore, apo A-I is closely related to HDL-C concentration. Proteomics research continues to expand apolipoprotein spectrum and other related protein components in HDL [4]. HDL lipids are mainly free or esterified fatty acids, phospholipids and different ceramides and sphingolipids [5]. Unlike other apolipoproteins, HDL exists as apolipoprotein precursor particles rather than mature apolipoproteins. These particles are mostly disk-shaped and consist of phospholipid bilayers and apolipoprotein stabilized unesterified cholesterol [6]. There are many subgroups of HDL, which are classified by volume: HDL3c, HDL3b, HDL3a, HDL2a, HDL2b; According to density, there are HDL2 (d = 1.063 - 1.125 g/ml), HDL3 (d = 1.125 - 1.21 g/ml), etc. [7].

2.2. Synthesis and Decomposition of HDL and Its Function-Related Proteins

The HDL subgroups increase or eliminate the neutral lipids, phospholipids in the components under the reaction of related proteins to undergo reconstruction or transformation, and proteins involved in HDL synthesis and decomposition and function-related include:

2.2.1. Serum Amyloid A (SAA)

SAA competitively combines with apo A-I and HDL, and can become the main protein component of HDL. HDL containing SAA mediates the decrease of cholesterol outflow activity [8] and is easier to be cleared by kidney. It has been reported that SAA has a certain role in the development of plaque.

2.2.2. CETP

CETP mediates the exchange of cholesteryl esters in HDL with triglyceride-rich lipoproteins to promote the reverse transport of cholesterol, so that the increase of TG component in HDL increases the catabolism and leads to a decrease in HDL-C concentration.

2.2.3. Endothelial Lipase (EL)

EL is consistent with liver lipase and lipoproteinase, and tends to hydrolyze triglyceride-rich lipoproteins. Current experimental studies have shown that overexpression of adenovirus gene into EL reduces the cholesterol component and phospholipid component of HDL.

2.2.4. Lecithin: Cholesterol Acytransferase (LCAT)

LCAT needs to transfer sn-2-acyl of lecithin to cholesterol under the action of apo A-I co-activation factor, so as to convert free cholesterol in HDL into cholesteryl ester. This process retains the core of HDL particles, and converts naive HDL into spherical particles, which is especially important for reverse cholesterol transport process.

2.2.5. Paraoxonase (PON)

PON1 is an enzyme related to HDL which can protect LDL from oxidation as well as an important mechanism for HDL to resist atherosclerosis. The reduction of PON1 can reduce HDL antioxidant function [9]. Moreover, HDL-PON can decompose peroxides on the surface of cell membrane. Some researchers have found that there is a negative correlation between HDL-PON activity and HDL peroxide level, which proves that PON not only reduces LDL and membrane oxidation, but also reduces HDL oxidation.

2.2.6. ATP-Binding Cassette Transporter A1 and G1 (ABCA1/ABCG1)

ABCA1 plays an important role in reverse cholesterol transport and HDL synthesis. ABCA1 promotes cholesterol efflux process (at least lipid apo A-I from cells) by consuming ATP to participate in cholesterol reverse transport process. The role of ABCG1 is to re-esterify apo A-I produced by ABCA1 pathway through energy consumption mediated lipid outflow.

2.3. HDL—Cholesterol Reverse Transport

Apo A-I, the skeleton of HDL protein, is mainly synthesized and secreted into circulation by liver and intestinal tract, and combines free phospholipids, cholesterol and triglycerides to form juvenile HDL particles [10]. Juvenile HDL is bound to the surface of peripheral tissues such as macrophages ABCA1 activates

intracellular cholesterol ester hydrolase to produce free cholesterol, which is transported to juvenile HDL [11], and forms cholesterol ester after LCAT catalysis, making it easier to embed into HDL core. This process converts low-fat HDL precursor particles into lipid-rich HDL (mature HDL) [12]. Subsequent mature HDL is released into the circulation and reaches the liver in combination with scavenger receptor-B1 (SR-B1), making HDL lipid components more susceptible to hydrolysis by lipases such as hepatic lipase to unload their lipid components [13]. Subsequently, lipid-poor HDL is released into the circulation to repeat the above-mentioned cholesterol transport process [14], which is the reverse transport of cholesterol—HDL removes excess cholesterol from the periphery and transports it to the liver for metabolism or to steroid synthesis organs for steroid hormonessynthesis. Thus, HDL prevents lipid toxicity and reduces the formation of foam cells, thereby reducing the formation of atherosclerotic plaque and reducing the risk of cardiovascular events [15].

3. HDL, Diabetes Mellitus and Cardiovascular Diseases

Cardiovascular diseases are still the leading cause of death in many developed and developing countries [12]. HDL's reverse cholesterol transport, anti-inflammatory [16], antioxidant and other functions play a great role in cardiovascular protection [17]. Type 2 diabetes mellitus and its metabolic syndrome affect HDL concentration and function in many ways.

3.1. Changes in Anabolism and Catabolism

Changes in anabolism and catabolism of HDL are accompanying insulin resistance, resulting in HDL synthesis with little triglyceride and little cholesterol ester, non-enzymatic saccharification of apo A-I and other HDL related proteins, oxidative modification of HDL lipids, apolipoproteins and enzymes, etc. [18].

3.1.1. Proteomic Changes

Chronic inflammation in patients with type 2 diabetes mellitus increases SAA [19]. SAA replaces apo A-I from HDL surface, and SAA-rich HDL is more susceptible to hydrolytic metabolism. Oxidative stress in patients with diabetes mellitus is increasing, resulting in a decrease in HDL-related proteins apo A-I and PON1.

3.1.2. Lipid Histological Changes

Insulin resistance and T2DM can increase the level of TG. Hypertriglyceridemia increases CETP-mediated transesterification of TG-rich lipoprotein and HDL cholesterol, which increases the content of TG-rich HDL (poor stability and loose binding with Apo-A1) in circulation and accelerates renal excretion [20]. Phospholipids in T2DM have changed, and the ratio of sphingomyelin/lecithin (the main determinant of HDL antioxidant function) has increased.

3.2. Functional Changes

The function of HDL depends on the shape of HDL (spherical shape is better

than discoid shape [21]), the size and composition of HDL (fatty acid composition/triglyceride content [22], glycated protein, peroxide protein).

3.2.1. Impaired Reverse Cholesterol Transport Function

ABCA1 and ABCG1 are key components in cholesterol reverse transport. Their expression in macrophages up-regulates lipid loading [23], while ABCA1 mediates cholesterol and phospholipid efflux to apo A-I, and phospholipids and cholesterol in mature HDL are mainly derived from ABCG1-mediated lipid efflux [24]. Mauldin *et al.* [25] demonstrated that hyperglycemia resulted in decreased ABCG1 expression and impaired function of macrophages in a mouse model of type 2 diabetes mellitus. *In vitro* experiments, RAW264.7 macrophages and HepG2 hepatocytes were incubated in unsaturated fatty acid medium, and the expression of ABCA1 mRNA and protein were both down-regulated [26]. Significantly elevated unsaturated fatty acids in patients with diabetes mellitus have been shown to phosphorylate ABCA1 through phospholipase D2 pathway and reduce its stability, thus affecting its function [27] and reducing cholesterol reverse transport. The increased SAA in T2DM patients binds to HDL and increases its affinity to macrophages by 3 to 4 times, while the affinity to hepatocytes decreases. The difference in affinity may lead to HDL switching from liver to macrophage clearance, which may be closely related to HDL's anti-atherosclerosis and anti-inflammatory properties changing to atherosclerosis promoting properties [8]. In addition, SAA has been shown to reduce HDL subpopulations and promote cholesterol outflow [28]. Advanced glycation products (AGEs) formed in patients with diabetes mellitus are increased, and AGE precursors hinder ABCA1-dependent intracellular cholesterol efflux [29]. In addition, AGEs [30] were also detected from lipoprotein isolated from diabetic subjects. The increase in apo A-I carbonyl modification associated with AGEs [30] may be related to abnormal HDL metabolism. A recent study shows that inflammation destroys many components of HDL cholesterol transport and efflux function [31]. Inflammation-induced down regulation of bile transporters ABCG5, ABCG8 and ABCB11 in the liver results in impaired cholesterol metabolism through the liver. The above-mentioned various mechanisms damage the reverse cholesterol transport process.

3.2.2. Impaired Antioxidant Function

Chronic inflammation induces the secretion of myeloperoxidase (MPO), and MPO activity increases in HDL in patients with type 2 diabetes mellitus [32]. MPO has been proved to selectively catalyzenitrosation, chlorination, oxidation of Apo-A1, and oxidized apo A-I selectively inhibit ABCA1-dependent cholesterol outflow process [33]. Apo A-I has a pro-inflammatory effect after oxidation by MPO. Oxidative modification of HDL apolipoproteins and enzymes in patients with diabetes [22] and increased sphingomyelin/phosphatidylcholine ratios lead to an increase in the surface hardness of HDL (a key antioxidant factor [34]), which reduces their antioxidant activity. The content of very low density

lipoprotein increases in type 2 diabetes mellitus, which is proved to be an effective LCAT activity inhibitor [35]. In addition to its importance in reverse cholesterol transport, LCAT can hydrolyze platelet activating factor (PAF) [36] [37] and phosphatidylcholine produced during lipoprotein oxidation, eliminating its pro-inflammatory function and cytotoxicity. In addition to its antioxidant properties, LCAT has an effect on the concentration of PON1 and PAF acetylhydrolase [38]. This indicates that LCAT plays an important role in the reverse cholesterol transport and HDL antioxidant function.

The anti-atherosclerosis effect of HDL is closely related to the regulation of LDL and cell membrane oxidation by PON1. Increased oxidative stress in T2DM reduces the concentration of PON1 [39], which is 40% of that in non-patients with diabetes mellitus [40]. PON1 inhibits the production of monocyte chemotactic protein 1 (MCP1) mediated by oxLDL through endothelial cells [41]. MCP1 induces monocytes to recruit subcutaneously, which lays a foundation for the occurrence of atherosclerosis. The protective effect of PON1 transfected cells and PON1 transgenic mice on macrophage oxidative stress [42] reduces the formation of atherosclerotic lesions [43], and PON1 deficiency is found to be related to macrophage oxidative stress and increased atherosclerosis [44]. The more peroxide components in HDL, the lower HDL-PON activity [45]. In short, the decrease of PON activity and HDL anti-membrane and LDL oxidation protection in patients with diabetes mellitus accelerates atherosclerosis in patients with diabetes mellitus.

3.2.3. Impaired Endothelial Protective Function

Some studies have found that the protective effect of HDL on vascular endothelium is obviously impaired in patients with T2DM [32]. Perségol *et al.* [46] proved that HDL lipid peroxidation and myeloperoxidase (MPO) activity increased in patients with T2DM, and the ability of resisting oxidized low density lipoprotein to inhibit endothelium-dependent vasodilation was impaired. Increased MPO can increases endothelial dysfunction [47] [48] and coronary heart disease risk [49]. *In vitro* studies have shown that HDL isolated from healthy individuals incubates endothelial cells and found that HDL could induce the expression of endothelial NO synthase (eNOS). However, the loss of HDL vasodilation function in patients with T2DM is due to the reduction of NO production and endothelium-dependent relaxation function. Moreover, in patients with diabetes and coronary heart disease, HDL's function of inhibiting expression of endothelial intercellular adhesion molecule-1 (VCAM-1) is absent, thus macrophages are more likely to adhere to activated vascular endothelial cells [47], and endothelium is more likely to be damaged. Recent studies have shown that HDL endothelial protective function mainly increases the number of serum endothelial progenitor cells (EPCs) [50] and accelerates endothelial cell neogenesis. Some studies have found that HDL loss from T2DM patients with coronary heart disease inhibits endothelial cell apoptosis because it cannot activate anti-apoptotic proteins and stimulate the pro-apoptotic pathway. *In vitro*, cardi-

omyocytes are incubated with high glucose, and HDL supplementation can offset the apoptosis-promoting effect of some high glucose on cardiomyocytes. In healthy control group and patients with diabetes mellitus, low HDL is an independent risk factor for endothelial dysfunction [51].

4. Diabetes Mellitus and Coronary Heart Disease

Numerous epidemiological studies have shown that diabetes is one of the independent risk factors for cardiovascular diseases. Endothelial dysfunction plays an important role in the pathogenesis of microangiopathy in patients with diabetes mellitus [52], and is the main cause of diabetes morbidity and mortality. At the early stage of atherosclerosis formation, endothelial dysfunction already exists, while there is no obvious morphological change in vascular wall at this time. Endothelial dysfunction is common in patients with diabetes mellitus. eNOS expression is down-regulated, NO synthesis is reduced, and endothelium-dependent vasodilation is abnormal [53]. Diabetes-related hyperglycemia and increase of angiotensin II (Ang II) can increase reactive oxygen species (ROS) in circulation. Oxidative stress causes endothelial dysfunction [54], partly due to NO degradation. NO has important antithrombotic, anti-apoptosis and anti-inflammatory functions. This makes people wonder whether other functions such as anti-inflammatory function and anti-apoptosis function are also changed after vascular endothelial relaxation dysfunction. A large number of studies have shown that activation of angiotensin II type 1 receptor (AT1R) increases vascular endothelial oxidation products and initiates apoptosis process, which promotes the occurrence and development of vascular endothelial dysfunction [54]. The expression level of AT1R determines its biological activity. Currently, several known agonists include glucose, angiotensin II, insulin, reactive oxygen species and others including diabetes itself [53]. The understanding of up-regulation of AT1R expression and angiotensin II mediated signaling in patients with diabetes mellitus comes from the discovery that endothelial function is repaired after patients with diabetes mellitus use AT1R antagonist [55]. Some studies have found that endothelial progenitor cells (EPCs) are also targeted to repair damaged sites after vascular wall integrity is destroyed, in addition to repairing adjacent mature endothelial cells [56]. EPC is not only reduced in number but also impaired in function in patients with diabetes mellitus [57], which may be related to endothelial dysfunction [58]. Primary myocardial injury in patients with diabetic heart disease occurs before hypertension and coronary artery disease. Ventricular dysfunction in diabetic heart disease is mainly due to myocardial cell apoptosis, interstitial inflammatory reaction, myocardial cell hypertrophy, glycogen accumulation in myocardium, changes in myocardial extracellular matrix (interstitial and perivascular fibrosis), myocardial microvascular lesions, intracellular Ca^{2+} abnormalities and endothelial cell dysfunction [59]. Some animal studies have found that cell apoptosis increases in diabetic animals induced by streptozotocin (STZ) [60], as well as in patients with di-

abetes mellitus [61]. Apoptosis is mainly due to increased oxidative stress caused by hyperglycemia. Hyperglycaemia not only increases the production of reactive oxygen species, but also inhibits the production of antioxidant enzymes [62]. In addition to inducing lipid peroxidation, ROS can also change cell proteins and initiate various stress signal pathways, such as Erk, JNK and p38 MAPK. Activation of cardiac p38 MAPK is of pathological importance in diabetic heart disease, which indicates that inhibition of p38 MAPK can improve STZ-induced left ventricular dysfunction in diabetic mice [63]. It has been confirmed in transgenic and knockout animal models that antioxidant enzymes superoxide dismutase (SOD), SOD-1, SOD-2, and extracellular (ec)-SOD convert O_2-anions into oxygen molecules and hydrogen peroxide [64], and SOD-2 is the same in patients with diabetes mellitus [65]. In the heart, overexpression of ec-SOD reduces macrophage infiltration and fibrosis, and improves left ventricular dysfunction [64], while up-regulation of SOD-2 expression can prevent apoptosis induction and protect mitochondrial respiratory function [66].

5. Conclusion

To sum up, HDL has long been considered as a protective component of cardiovascular diseases, and HDL-C concentration is a good predictor of cardiovascular disease risk factors. Type 2 diabetes mellitus affects HDL metabolism and thus reduces HDL-C concentration, but increasing HDL-C concentration with drugs has not been able to reduce the risk of cardiovascular events as scheduled. Not only that, T2DM changes HDL in size, shape and composition, thus changing its function and even transforming it from cardiovascular protective effects such as anti-inflammation, anti-apoptosis and antithrombotic to cardiovascular disease events such as pro-inflammation, pro-apoptosis and thrombosis. Therefore, we should not only pay attention to HDL-C concentration, but also pay attention to its morphology, composition and functional changes in the treatment of type 2 diabetes patients with cardiovascular diseases, which provides a new research direction for cardiovascular disease risk prediction and treatment targeting of patients with T2DM.

Conflicts of Interest

The authors declare no conflicts of interest regarding the publication of this paper.

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Virilizing Ovarian Leydig Cell Tumor with Multiple Non-Functional Endocrine Neoplasias: A Case Report

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Abstract

Ovarian Leydig cell tumor, a sub-type of ovarian steroid cell tumor, accounts for less than 0.1% of all ovarian tumors. It can affect women of any age group but is most common in postmenopausal women. We here report a case of virilizing ovarian Leydig cell tumor with multiple non-functional endocrine neoplasias (pituitary and adrenal adenomas) in a 48-year-old woman. She first presented with sub-abdominal pain and hirsutism since menopause three years ago. Subsequently, she had slight facial acne, voice deepening, breast atrophy, and a prominent Adam's apple. Her hormone profile showed an elevated level of testosterone, high free androgen index, low levels of luteinizing hormone and follicle stimulating hormone, and normal levels of random cortisol, androstenedione, 17-hydroxyprogesterone and dehydroepiandrosterone sulfate. A pelvic enhanced magnetic resonance imaging (MRI) scan showed nodules in the right ovary, and a pituitary enhanced MRI revealed a microadenoma. An enhanced computerized tomography scan of the adrenal gland revealed left adrenal nodules, possibly adenomas. After a right cystectomy and right fallopian tube resection, her testosterone level declined to 0.38 nmol/L and the symptoms associated with hyperandrogenism improved. This is a rare case of virilizing ovarian Leydig cell tumor with multiple non-functional endocrine neoplasias. We believe our findings will be helpful in the clinical diagnosis and treatment of hyperandrogenism.

[#]Xiaohong Xu and Xiaoxiao Song contributed equally to this work.

Keywords

Leydig Cell Tumor, Ovary, Adrenal Adenomas, Hyperandrogenism

1. Introduction

Leydig cell tumor is a rare subtype of the ovarian sex cord-stromal tumors, composed of Leydig cells [1]. It accounts for less than 0.1% of all the ovarian tumors. The clinical features of Leydig cell tumors are associated with hormone levels and mass occupancy effects. It is characterized by a wide range of age of onset, low malignancy rate, and good prognosis after surgical resection. Ovarian Leydig cell tumors often occur in postmenopausal women. Based on existing case reports, Leydig cell ovarian tumors accompanied by an adrenal adenoma can be difficult to diagnose [2]. We here report a unique case of virilizing ovarian cell tumor accompanied by multiple non-functional endocrine neoplasias. The patient had non-functional pituitary microadenomas as well as adrenal adenomas. She also had a thyroid adenoma which was resected 30 years ago. This is a rare case of an ovarian Leydig cell tumor with multiple non-functional endocrine neoplasias.

2. Case Report

A 48-year-old woman visited a local hospital for sub-abdominal pain and hirsutism since she had menopause three years ago. She was pregnant only once and gave birth to one child. The initial laboratory findings showed an elevated testosterone level (value not available), but she did not receive any treatment. She had laser hair removal performed several times for excessive hair growth. She went to the hospital for a re-examination two months ago, and the hematological tests revealed a high level of testosterone (>35 nmol/l). Additionally, a pituitary enhanced magnetic resonance imaging (MRI) showed microadenomas (size was not marked). She then came to our hospital for treatment.

The patient had undergone a partial left thyroidectomy for a thyroid adenoma, 30 years ago and was receiving a long-term oral administration of Euthyrox (75 μg QD). Uterine myomectomy was performed more than 10 years ago.She had achieved menopause at the age of 45 with no postmenopausal bleeding. She had a normal menstrual history before menopause no history of hypertension and diabetes. She did not smoke or drink. A physical examination revealed the following: weight: 60 kg and body mass index (BMI): 22.4 kg/m^2. She presented with normal hair distribution because of the laser hair removal. However, she had slight facial acne, voice deepening, breast atrophy, and a prominent Adam's apple.

The hormone profile of the patient revealed the following: testosterone: 29.42 nmol/L (reference value < 2.5), free androgen index: 79.63 (0.3 - 9.6), luteinizing hormone (LH): 0.86 IU/L (11.30 - 39.80), follicle stimulating hormone (FSH):

5.02 mIU/L (21.7 - 153), estradiol E2: 203.5 pmol/L (post menopause, <110), thyroid peroxidase antibody: >1000 IU/ml (<5.61), thyroglobulin antibody: 14.7 (<4.11), and thyroglobulin: 129.20 μg/L (3.5 - 77.0). Random cortisol, androstenedione, 17-hydroxyprogesterone and dehydroepiandrosterone sulphate (DHEA-S) concentrations were within the normal range.

A pituitary enhanced MRI revealed abnormal nodular signals in the posterior pituitary suggestive of microadenomas or Rathke cysts (**Figure 1**). An enhanced computerized tomography scan of the adrenal gland showed left adrenal nodules which were diagnosed as adenomas (**Figure 2**). Transvaginal B-mode ultrasonography showed no significant abnormalities in the ovaries.

A medium dose dexamethasone suppression test resulted in significant inhibition of cortisol and adrenocorticotropic hormone (ACTH), while just a 4% decrease in the serum levels of testosterone (baseline level 37.44 nmol/L, after test 35.97 nmol/L), suggesting that the hyperandrogenism may not be due to the adrenal gland. To further clarify whether the high concentration of testosterone originated from the proliferative left adrenal gland and to determine the location of the lesion, bilateral adrenal venous blood sampling was performed. However, the adrenal venous blood collection was unsuccessful.

A pelvic enhanced MRI scan showed a right ovarian nodule (14.3 * 28.4 mm), and multiple uterine fibroids (**Figure 3**). To detect other possible neoplasms and explore the source of the abnormal testosterone secretion, she had a whole-body positron emission tomography (PET) scan. The findings revealed a low-density lesion (16.6 * 20.8 * 24.8 mm) in the right ovary, bilateral thyroid nodules and possibly a left adrenal adenoma. Based on the above results, the hyperandrogenism appeared to be arising from the right ovary.

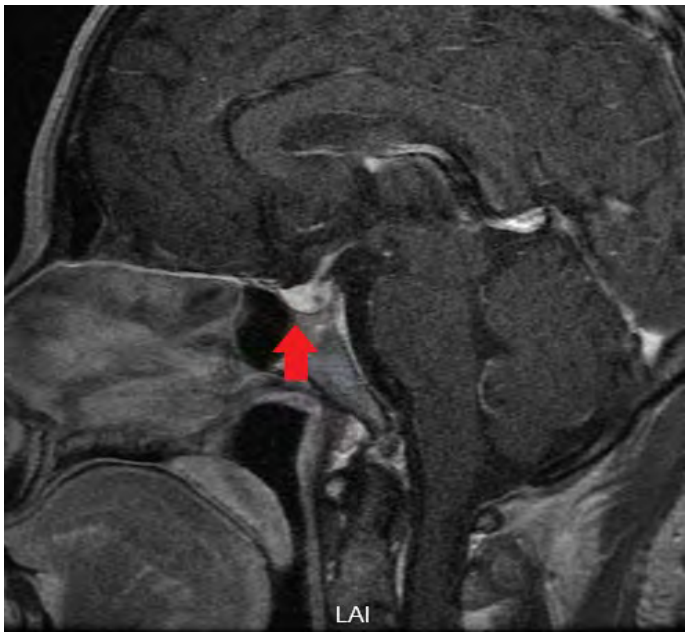

Figure 1. Pituitary MRI enhancement findings (sagittal): Hypointense nodules between the anterior and posterior pituitary glands.

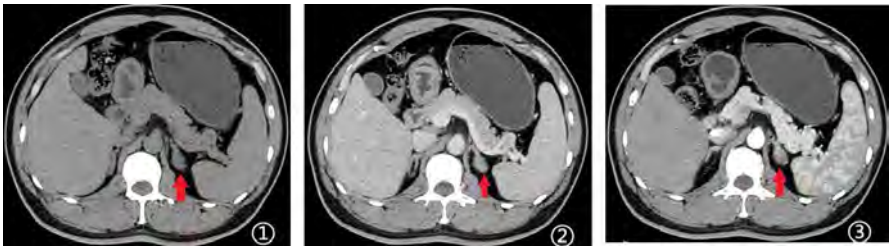

Figure 2. Adrenal CT enhancement findings: (1) CT scan of the adrenal gland, Adrenal CT enhancement in the (2) venous, and (3) arterial phases.

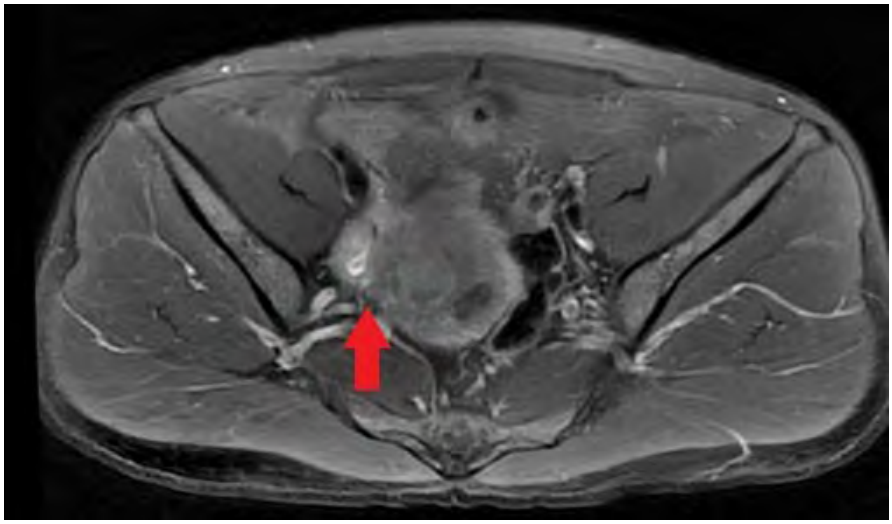

Figure 3. Pelvic MRI findings (T2W2): In the right appendix area, is a lesion with unclear local uterine boundary, and slightly high signal intensity.

The patient went to another hospital for surgical treatment on October 25, 2017. She had a right cystectomy, right fallopian tube resection, and adenomyoma debridement, but no adjuvant chemotherapy. During surgery, the right ovary was found to be enlarged with a cystic mass of 2*2 cm. The postoperative pathological evaluation of the right ovarian cyst indicated an ovarian Leydig cell tumor combined with an inclusion cyst. A sex hormone test on the fourth day after surgery reported a significant decrease in testosterone level (0.38 nmol/L), increase in LH (13.49 IU/L) and FSH (23.5 IU/L) to normal levels, and normal level of the concord hormone (0.19 ng/ml). After 14 months of surgery, the serum testosterone level has been normal, and the signs and symptoms of hyperandrogenism including the voice change, facial acne, breast atrophy, and Adam's apple have all improved.

3. Discussion

Hyperandrogenism and hirsutism are mostly related to polycystic ovary syndrome (PCOS). However, less than 5% of the cases are caused by androgen-secreting tumors of either adrenal or ovarian origin [3]. Serum testosterone levels are typically used to assess androgen levels. Androgen-producing tumors

should be considered when patients present with rapid progression of signs and symptoms of hyperandrogenism, especially when the testosterone levels are more than three times the upper reference limit [4]. DHEA-S is a marker of adrenal androgen production, and serum DHEA-S levels greater than 16 umoL usually point to an androgen-secreting adrenal tumor [5]. In Leydig cell tumors, serum testosterone levels are expected to be slightly elevated [6] or highly elevated as seen in our case. The patient, this case, was suspected of having androgen-secreting neoplasms due to the very high level of testosterone (37.44 nmol/l). The normal transvaginal ultrasonography findings and the adrenal adenoma led us to consider adrenal-derived hyperandrogenism. However, the normal serum level of DHEA-S and the PET scan results ruled out the adrenal cause.

It is noteworthy that in this case dexamethasone was used to exclude congenital adrenal hyperplasia (CAH), rather than the ACTH excitation test, which is commonly used worldwide. CAH is a group of autosomal recessive hereditary diseases [7] that affects the adrenal glands. It is caused due to the deficiency of an enzyme, leading to a partial or complete block of cortisol synthesis. This, in turn, results in an increase in corticotropin-releasing hormone (CRH) secreted by the hypothalamus and ACTH secreted by the pituitary, which stimulates adrenocortical hyperplasia, thereby resulting in varying degrees of adrenocortical dysfunction. The most common cause of CAH is 21 hydroxylase deficiency (21OHD) [7], followed by 11 beta-hydroxylase deficiency [8]. Rapid ACTH excitation test is recommended for differential diagnosis in clinical practice [9], though it is rarely used in China due to the lack of ACTH drug sources. Dexamethasone inhibits cortisol and adrenal-derived androgen secretion by inhibiting the pituitary ACTH secretion. Therefore, since the 1980s, China has been using a functional test to differentiate androgen sources by giving moderate doses of dexamethasone [10], to detect changes in levels of ACTH, 17-OHP and total testosterone.

Among the ovarian androgen-secreting neoplasms, steroid cell tumors are quite rare [11]. Ovarian Leydig cell tumor, a sub-type of the ovarian steroid cell tumors, accounts for less than 0.1% of all ovarian tumors. It can affect women of any age group, but is most common in postmenopausal women [12] [13]. Over 75% of the cases of ovarian Leydig cell tumors present with severe hyperandrogenism characterized by hirsutism, secondary amenorrhea, virilization, and a small number of tumors with high estrogen or non-endocrine function. Likewise, nearly 75% of the cases with severe hyperandrogenism involve Leydig cell tumors [14]. More than 95% of the ovarian Leydig cell tumors are unilateral, and only 7 bilateral cases have been reported. Most of these tumors are benign and smaller than 4 cm in size [15]. Surgical removal of these tumors results in significant improvement in the symptoms and has an excellent prognosis.

The strategies for managing Leydig cell tumors include surgery and adjuvant chemotherapy (usually used in malignant tumors).The type of surgery which includes unilateral (for fertility preservation), bilateral salpingo-oophorectomy or

cystectomy, usually depends on the patient's age, fertility requirements, and the nature of the tumor. Conservative and fertility-sparing surgery is especially recommended in younger patients or patients without children.

Due to the lack of clear diagnostic criteria, the diagnosis of ovarian Leydig cell tumors still remains challenging and cannot be done without surgery. Moreover, due to their small size and density, these tumors are usually invisible on ultrasonography and CT. When accompanied by an adrenal adenoma, the diagnosis becomes even more difficult. Though hyperandrogenism due to an adrenal adenoma is not uncommon, it can be very difficult to detect the origin of the hyperandrogenism, especially with high levels of cortisol [2]. Ovarian and adrenal venous sampling can be performed, but both of them require advanced technology, and the success rate is low.

4. Diagnostic Process

Based on this case, we reviewed the existing literature and summarized what is known about the etiology and diagnosis of hyperandrogenism (**Figure 4**).

The main clinical manifestations of hyperandrogenism include hirsutism, acne, androgenic alopecia, masculinization and some special manifestations [16] [17]. Clinically, the etiology of hyperandrogenism is complex, and PCOS is the most common functional etiology [18]. The organic etiologies mainly include congenital adrenocortical hyperplasia, androgen-secreting tumors, and abnormal sexual differentiation [16]. Therefore, the key to the diagnosis of hyperandrogenism is to determine the source and etiology of androgen production [19]. If the patient had a previous history of abnormal menstruation, the lesion might have originated from the ovary [17]. On the other hand, if the patient developed hirsutism and masculinization in a short time, androgen-producing adrenal or ovarian tumors should be considered [3]. Though the main manifestation of idiopathic hirsutism includes excessive hair growth and normal ovulation, the related medication history and stress factors such as menopause and pregnancy also help in making the diagnosis [5]. Based on the patient's medical history, physical examination, B-mode ultrasound, CT and MRI, large ovarian, adrenal or pituitary tumors can be excluded, which can then provide diagnostic clues for unexplained hyperandrogenism [20]. When the tumor is small and concealed, it is easy to miss in B-mode ultrasound and other imaging examinations. If the patient suffered from both adrenal mass and ovarian mass, location diagnosis is very difficult [21]. Adrenal venous blood collection or ovarian venous blood collection technology is of critical significance in the differential diagnosis of androgen source. Determination of hormone levels is essential for the diagnosis of hyperandrogenism [22]. Increase in different kinds of androgens may indicate the presence of lesions and therefore, can provide important leads for clinical diagnosis. Elevated levels of testosterone and LH/FSH > 2 are suggestive of PCOS. Adrenal tumors are characterized by marked elevation of testosterone with DHEA-S > 16 μmol/L. In cases of congenital adrenocortical hyperplasia,

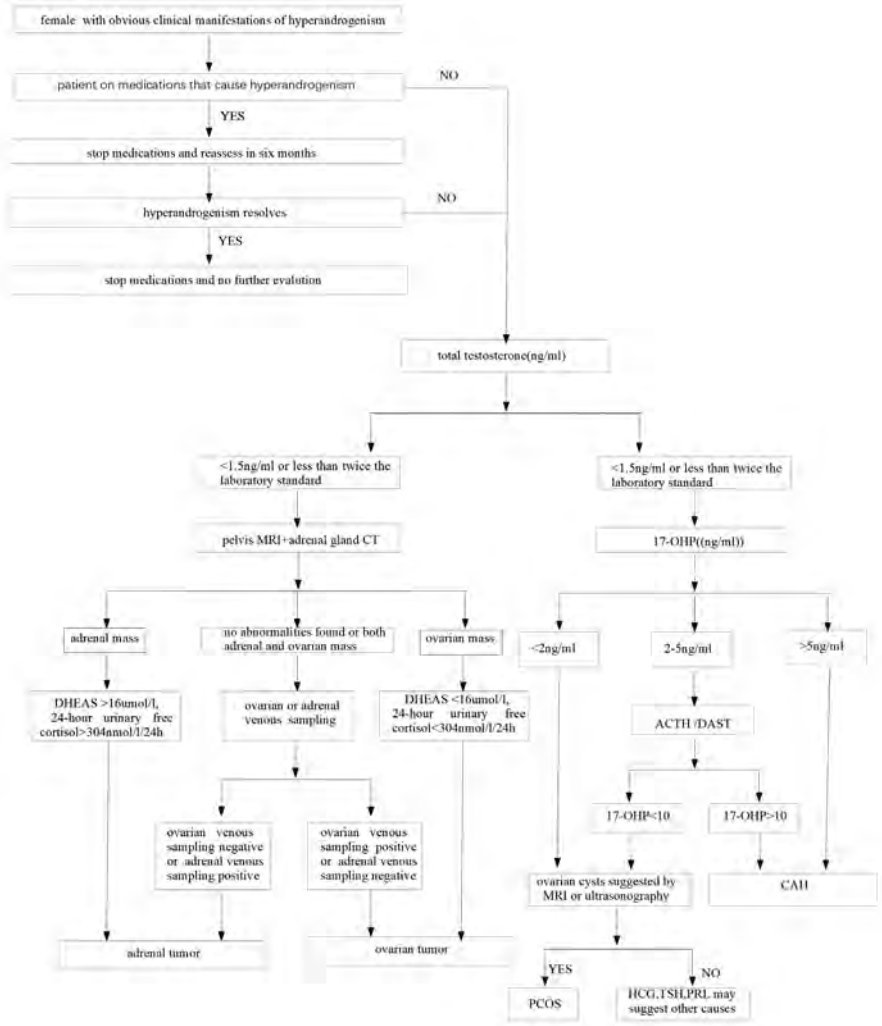

Figure 4. Diagnostic process of hyperandrogenism.

testosterone and 17-OHP are elevated, or markedly elevated after adrenocorticotropic stimulation [23] [24]. The elevation of dihydrotestosterone with low testosterone suggests the possibility of idiopathic hirsutism [25] [26]. When testosterone and cortisol levels are elevated simultaneously, adrenocortical hyperfunction should be evaluated using the dexamethasone inhibition test [27] [28] [29].

5. Conclusions

In conclusion, we report a case of ovarian Leydig cell tumor with multiple non-functional endocrine neoplasms in post-menopausal women, characterized by hirsutism. This is a rare case of a Leydig cell tumor with multiple non-functional endocrine neoplasms, though Leydig cell tumors accompanied by adrenal adenomas are not uncommon.

Although rare and difficult to diagnose, ovarian Leydig cell tumors should be considered in cases with severe hyperandrogenism and hirsutism after the exclu-

sion of adrenal-derived hyperandrogenism, especially in postmenopausal women. In such patients with hirsutism and significantly elevated testosterone level, an oophorectomy should be considered after the exclusion of adrenal causes. In our case, surgical intervention confirmed the final diagnosis of a Leydig cell tumor. As expected, following surgery, the hormone levels returned to normal and clinical symptoms of hyperandrogenism improved.

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Conflicts of Interest

The authors declare no conflicts of interest regarding the publication of this paper.

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