

Association of HELLP Syndrome With Autoimmune Antibodies and Glucose Intolerance

RAIMUND WEITGASSER, MD
DIETMAR SPITZER, MD
ILSE KARTNIG, MD

MICHAEL ZAJC, MD
ALFONS STAUDACH, MD
FRIEDRICH SANDHOFER, MD

OBJECTIVE — HELLP syndrome is a severe form of preeclampsia, characterized by hemolysis (H), elevated liver enzymes (EL), and low platelets (LP), whose pathogenesis is unclear. Autoimmunity is thought to play an important role. After the observation of development of type 1 diabetes in a patient with HELLP syndrome, we assumed a possible disease association based on autoimmune reactions.

RESEARCH DESIGN AND METHODS — We examined 70 women with HELLP syndrome for the presence of autoimmune markers and glucose intolerance. Free thyroxine, triiodothyronine, thyroid-stimulating hormone, anti-thyroglobulin antibodies, thyroperoxidase antibodies, thyrotropin receptor antibodies, antinuclear antibodies (ANAs) and anti-DNA, islet cell antibodies, GADA, an oral glucose tolerance test, and HbA_{1c} were determined postpartum. Patients with positive autoimmune markers or glucose intolerance were prospectively followed and repeated testing was performed. There were 60 women with a normal course of pregnancy matched for age, BMI, and number of pregnancies, which served as a control group.

RESULTS — From the HELLP patients, 22 (31%) compared with only 6 (10%) control subjects had autoimmune antibodies ($P < 0.01$). There were 16 HELLP patients (23%) who exhibited only 1 kind of autoantibody (5 ANA, 9 thyroid antibodies, and 2 GADA), whereas in 6 HELLP patients (8.5%) 2 different antibodies were found. In all but 4 patients of the study group, these antibodies disappeared during 3 ± 1.5 years of follow-up. Glucose intolerance was detected in 22 (31%) of the HELLP patients, 17 of them had impaired glucose tolerance (IGT), and 5 had diabetes, whereas only 4 subjects (6.5%) with IGT at postpartum were found in the control group ($P < 0.01$). During the follow-up, 2 HELLP patients were still diabetic and another 2 HELLP patients (1 GADA positive) had IGT versus 1 control subject.

CONCLUSIONS — Our data give evidence that HELLP syndrome is associated with various autoimmune antibodies and glucose intolerance. Because glucose intolerance and/or autoimmune markers persisted during long-term follow-up in 6 patients with HELLP syndrome versus 1 in the control group, it may become advisable to reexamine patients with HELLP syndrome for detection of diabetes and autoimmune disorders.

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From the First Department of Medicine (R.W., ES.), the Department of Obstetrics and Gynecology (M.Z., D.S., A.S.), and the Second Department of Medicine (I.K.), St. Johannes Spital, Salzburg General Hospital, Salzburg, Austria.

Address correspondence and reprint requests to Raimund Weitgasser, MD, First Department of Medicine, St. Johannes Spital, Salzburg General Hospital, Muellner Hauptstrasse 48, A-5020 Salzburg, Austria. E-mail: r.weitgasser@lksbg.gv.at.

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Abbreviations: ANA, antinuclear antibody; APCA, anti-parietal cell antibodies; GADA, GAD antibodies; ICA, islet cell antibody; IGT, impaired glucose tolerance; OGTT, oral tolerance glucose test; TGAB, thyroglobulin antibodies; TPAB, thyroperoxidase antibodies; TRAB, thyrotropin receptor antibodies; WHO, World Health Organization.

A table elsewhere in this issue shows conventional and Système International (SI) units and conversion factors for many substances.

HELLP syndrome is a rare but severe form of preeclampsia with an incidence of 1:250 pregnancies in our hospital, characterized by hemolysis (H), elevated liver enzymes (EL), and low platelets (LP) (1–5). Obstetricians advocate early detection of the disease to induce labor or perform a cesarean section for prompt recovery of the patient and normalization of symptoms and pathological findings (2–5). In patients with HELLP syndrome, autoimmune platelet antibodies and cytotoxic antibodies have been detected, thus making the presence of an autoimmune disorder most likely (6,7). In pregnancy, autoimmunity has been found to be related to reproductive failure and recurrent miscarriage (8–11). Highly abnormal levels of anti-phospholipid antibodies have been detected in pregnancies associated with maternal hypertension and fetal growth retardation (7,12). Antinuclear antibodies (ANAs) were found to occur more often in patients with preeclampsia, intra-uterine growth retardation, fetal death, or abruptio placentae (12,13). However, for patients with HELLP syndrome, data on autoimmunity are not conclusive so far. Because of the observation of development of type 1 diabetes in 1 of our patients with HELLP syndrome (14), we investigated a possible association of HELLP syndrome with autoimmune-related disorders. In our study, we report the findings of various autoimmune antibodies and glucose intolerance at postpartum in patients with HELLP syndrome. All patients positive for autoimmune antibodies or glucose intolerance at postpartum were followed prospectively for a mean of 3 years.

RESEARCH DESIGN AND METHODS

Patients

From all 74 patients who were admitted to our hospital with HELLP syndrome during the last 7 years, 4 women were excluded from further evaluation because of incomplete data in 3 cases and prednisone treatment before examination in 1 case. In the 70 patients who were eligible for the study,

Table 1—Clinical data of patients with HELLP syndrome and control subjects

	HELLP patients			Control subjects	P
	All	With autoimmune antibodies	With glucose intolerance		
n	70	22	22	60	—
Age (years)	28 ± 5	29 ± 5	29 ± 6	27 ± 5	NS
Pregnancies (n)	1.7 ± 1	1.2 ± 0.5	1.6 ± 1	1.6 ± 0.8	NS
Age of gestation (weeks)	33 ± 4.5	34 ± 4	33 ± 4.9	37.5 ± 3.5	<0.001
BMI (kg/m ²)	26.5 ± 3.3	25 ± 3.5	25 ± 2.6	26.7 ± 3.2	NS
AST (U/l)	226 ± 212	235 ± 148	234 ± 188	13 ± 6.9	<0.001
ALT (U/l)	206 ± 194	255 ± 165	198 ± 150	8 ± 4.1	<0.001
LDH (U/l)	889 ± 507	811 ± 396	927 ± 582	254 ± 77	<0.001
Platelets (×10 ⁹ /l)	60 ± 31	62 ± 35	62 ± 38	226 ± 54	<0.001

Data are n or means ± SD; P values given for control subjects against all HELLP patients. ALT, alanine aminotransferase; AST, aspartate aminotransferase; LDH, lactate dehydrogenase.

HELLP syndrome was diagnosed at our Department of Gynecology and Obstetrics according to the definition by Sibai et al. (2). Besides a physical examination directed toward signs of autoimmune disease, the history of collagen disease, thyroid disorder, or diabetes in the patient and her first-grade relatives was stated at postpartum.

Laboratory measurements

Routine laboratory parameters, especially blood cell count (Max M; Instrumentation Laboratories, Vienna), coagulation, and liver and renal function parameters, performed on a Hitachi automated analyzer (Roche Diagnostics, Mannheim, Germany), were determined daily (until values normalized) during each patient's stay in the obstetrics unit. The highest values for aspartate aminotransferase, alanine aminotransferase, and lactate dehydrogenase, and the lowest values for platelets measured were used to determine the patient's characteristics (Table 1). Seven to 14 days after initiation of labor or a cesarean section, the following laboratory measurements were performed: an oral glucose tolerance test (OGTT) with 75-g glucose load evaluated according to World Health Organization (WHO) criteria (14); plasma glucose by glucose oxidase method using a Beckman Analyzer 2 (Beckman, Palo Alto, CA); HbA_{1c} by high-performance liquid chromatography (HPLC Diamat; Bio-Rad, Hercules, CA) with a normal range in our laboratory of 4.2–6.0% (range 2 SD); islet cell antibodies (ICAs) by immunofluorescence method (16); for GAD antibodies (GADA), a radioimmunoassay (GAD-RIA; Brahm's Diagnostica, Berlin), normal range <7 mU/ml was used; free thyroxine (T4), normal range 0.8–1.8 ng/dl, and thyroid

stimulating hormone, normal range 0.5–3.5 mU/l, were measured by chemoluminescence method (Bayer Diagnostics, Leverkusen, Germany); and triiodothyronine (T3), normal range 3–7.5 pmol/l, was measured by electro-chemo-luminescence (Roche Diagnostics, Mannheim, Germany); for measurement of thyroglobulin antibodies (TGAB), normal range <100 U/ml, and thyroperoxidase antibodies (TPAB), normal range <100 U/ml, an immunoenzyme-assay (Pharmacia & Upjohn, Freiburg, Germany) was used; for thyrotropin receptor antibodies (TRAB), normal range <7 U/ml, a radioimmunoassay (Iason Labormedizin, Graz, Austria) was used; ANAs, IgG, and anti-parietal cell antibodies (APCA), positive with titers >1:40, were determined by indirect immunofluorescence method (Sanofi/Pasteur, Vienna, and Bios, Munich); and anti-DNA antibodies, normal range <25 U/ml, were measured by enzyme-linked immunosorbent assay (Elias Medizintechnik, Freiburg, Germany).

Control subjects

Sixty women with a normal course of pregnancy, matched for age, BMI (kg/m²), and number of pregnancies, served as a control group. Recruitment encompassed all women within a defined period of 3 months who were admitted to our Department of Gynecology and Obstetrics for delivery and who had given written consent for performance of the above-mentioned laboratory examinations. Patients with gestational diabetes, for whom there is a higher probability to develop diabetes during follow-up compared with normal pregnant women, were excluded from the control group. A selection bias between our study group and

the control subjects due to the difference in gestational age could be excluded as OGTTs were performed 1–2 weeks postpartum.

Follow-up

Patients with positive autoantibodies or diabetes at postpartum were prospectively followed and repeated testing was performed. Also, patients who were antibody negative but had IGT or diabetes were followed and tested again for the underlying disorder. Mean follow-up for all of these patients was 3 ± 1.5 years (range 0.8–6.8).

Statistical analysis

Comparison of data is given for patients with HELLP syndrome versus control subjects. Subgroup analysis was performed for patients with HELLP syndrome either with or without autoimmune markers. Patients with glucose intolerance were also evaluated separately. Data are given as means ± SD. Significance of differences between study groups has been determined by the use of the Mann-Whitney U test; P < 0.05 was considered significant.

RESULTS — Seventy patients with HELLP syndrome were included in our analysis. HELLP syndrome was diagnosed according to the criteria of Sibai et al. (2). All patients showed hemolysis, elevated liver enzymes, and low platelet counts (clinical data are presented in Table 1).

Postpartum findings

From the HELLP patients, 22 (31%) exhibited autoimmune antibodies: 16 (23%) had only 1 kind of antibody, 5 of them had elevated ANA titers, 2 had TGAB, 7 had TRAB, and 2 had GADA. In 6 patients (8.5%), 2 different autoimmune antibodies could be detected (Table 2). One patient (age 30 years), who had already developed a HELLP syndrome in 2 previous pregnancies, showed elevated ANA IgG of 1:160, APCA IgG 1:1280, TRAB 9 U/ml, and impaired glucose tolerance (IGT). From the 60 control subjects, autoimmune antibodies were found in only 6 women (10%): 3 had elevated ANA, 1 had elevated TGAB, and 2 had GADA. The difference in the frequency of antibodies between HELLP patients and control subjects was highly significant (P < 0.01). Three patients in the HELLP group had a history of Hashimoto thyroiditis, but had no thyroid antibodies at postpartum examination.

Glucose intolerance was diagnosed in 22 (31%) of the 70 patients with HELLP syndrome: 17 (24%) had IGT

Table 2—HELLP patients with autoimmune antibodies at postpartum

Patient, age (years)	Glucose intolerance	ANA titer	Thyroid antibodies (U/ml)			GADA (mU/ml)
			TGAB	TPAB	TRAB	
VR., 25		1:160	101			
G.G., 27		1:640			8	
M.G., 18		1:80				
M.R., 24		1:80				
S.M., 25		1:80				
E.F., 29		1:160				
G.S., 29	IGT	1:160				
E.G., 30	IGT	1:160			9	
U.R., 26					7	
S.K., 29					7	
R.M., 31	IGT				8	17
C.L., 37	D			232		10
E.W., 31	D		138			
E.E., 38	D		137			
T.B., 21	IGT				13	
H.R., 23	IGT				8	
T.M., 38					11	
A.S., 27			115	317		
M.W., 35					8	
I.L., 29					7	
R.P., 24						8
M.B., 29						550

D, diabetes.

and 5 (7%) had diabetes according to WHO criteria. Glucose values during the OGTT in these patients were as follows: fasting, 4.8 ± 1.1 ; at 1 h, 9.3 ± 1.4 ; and at 2 h, 8.8 ± 1.0 mmol/l. Of these HELLP patients with glucose intolerance, 8 were positive for autoimmune antibodies (ANA, TGAB, TPAB, TRAB, and GADA) (Table 2). In the control group, only 4 (6.5%) women had IGT and none had diabetes. OGTT glucose values in the 4 control patients with IGT are as follows: fasting, 3.5 ± 1.1 ; at 1 h, 9 ± 0.8 ; and at 2 h, 8.9 ± 0.5 mmol/l. The difference in the frequency of glucose intolerance

between HELLP patients and control subjects was significant ($P < 0.01$). Two HELLP patients with IGT and 2 patients without glucose intolerance had elevated GADA and all were ICA negative. One of the GADA-positive patients had developed a HELLP syndrome during her first and fourth pregnancies. HbA_{1c} was normal in all patients (4.8 ± 0.6 vs. $4.6 \pm 0.7\%$ in the control subjects, $P = NS$). The clinical data of HELLP patients with autoimmune antibodies or glucose intolerance taken separately were not different compared with data for all HELLP patients ($P = NS$) (Table 1).

For all other subjects (48 HELLP patients and 56 control subjects), values of the OGTTs were normal according to the WHO criteria. However, blood glucose values were significantly higher for HELLP patients compared with those for the control subjects (fasting glucose 4.6 ± 0.5 vs. 3.5 ± 0.7 mmol/l, $P < 0.001$; 1-h glucose 7.8 ± 1.6 vs. 6.6 ± 1.5 mmol/l, $P = 0.003$; 2-h glucose 6.3 ± 1.1 vs. 5.4 ± 1.0 mmol/l, $P < 0.001$).

Findings during the follow-up period

All patients with autoantibodies and/or glucose intolerance at postpartum were reexamined 6 months later. From the 22 patients with autoantibodies at postpartum, only 4 had autoantibodies 6 months later: 2 had GADA (18 and 500 mU/ml), 1 had TGAB (193 U/ml), and 1 had TPAB (991 U/ml). From the 22 patients with glucose intolerance at postpartum, only 2 had diabetes and 2 had IGT 6 months later. One of the 2 patients with diabetes was classified as type 1 diabetic with negative C-peptide; the other patient had type 2 diabetes, which was well controlled by diet (Table 3). BMI in these patients with glucose intolerance was normal (between 20–25 kg/m²); only 1 patient was overweight (C.L., age 37 years, BMI 30 kg/m²). All patients with autoantibodies or glucose intolerance 6 months postpartum remained positive in repeated follow-up examinations after another 6 months, and once a year thereafter.

In the control group, all 6 subjects with autoimmune antibodies at postpartum were negative 6 months later, and of the 4 subjects with IGT at postpartum, only 1 patient, with a BMI of 28 kg/m², had IGT 6 months later.

CONCLUSIONS — To date, the etiology and pathogenesis of the HELLP syndrome are not well understood. Gleicher

Table 3—Characteristics of all patients with persisting autoimmune antibodies or glucose intolerance during follow-up

Patients (n = 7)	Autoimmune antibodies, glucose intolerance	Patient's age (years)	Age of gestation (weeks)	Pregnancies (n)	BMI (kg/m ²)	Platelets ($\times 10^9$ /l)	AST (U/l)	Follow-up (years)
E.W.	Type 2 diabetes	31	38	1	25	9	152	5.0
G.L.	Type 1 diabetes	30	35	1	20	25	150	4.1
C.L.	IGT, TPAB	37	37	4	31	99	62	0.8
R.M.	IGT, GADA	31	35	1	22	46	420	1.0
M.B.	GADA	29	26	2	24	83	91	1.5
VR.	TGAB	25	36	1	25	15	618	3.0
S.V.	IGT	24	41	1	28	177	7	2.6

S.V., control subject; all others, HELLP patients. AST, aspartate aminotransferase.

(6,7) described the occurrence of antiplatelet and cytotoxic antibodies in patients with HELLP syndrome. To our knowledge, no other data with respect to antibodies have been reported for the HELLP syndrome. In our study, autoantibodies were found in 31% of the HELLP patients, whereas in the control group, only 10% had autoantibodies. The frequency of autoantibodies in our control subjects is higher compared with data from Rosenberg et al. (17) and Kiuttu et al. (18), who found ANA in 4 and 5%, respectively, of normal pregnancies and no difference compared with nonpregnant female control subjects. This finding is confirmed in a larger survey by Piura et al. (19). For higher-risk pregnancies, Matthiesen et al. (13) reported a significantly higher percentage of positive ANA. Our study shows that autoimmune phenomena, in particular of transient predominance, are a frequent finding in HELLP syndrome. Although in our HELLP patients only 6% had kept their autoantibodies 6 months postpartum, all of them stayed positive during further follow-up. It is presumed that autoimmune mechanisms play a role in the pathogenesis of the HELLP syndrome because the fetus acts as an antigen source for the mother and that transfer of fetal proteins to the mother may mediate autoimmunity, representing a subclinical rejection mechanism of the fetal allograft (6,20). Strong evidence for an autoimmune etiology of the HELLP syndrome is, on the other hand, derived from the observation of IgG antibody-mediated passive disease transfer to the fetus. It has been reported that up to 83% of infants born of mothers with HELLP syndrome show signs of hemolysis (bilirubinemia and abnormal peripheral blood smears with burr cells and/or schistocytes) and that 26% were thrombocytopenic at birth (6). In HELLP patients, comparable with predictive markers for type 1 diabetes such as ICA and GADA (21–24), the persistence of ANA and thyroid antibodies could lead to the development of collagen vascular or thyroid disorders later on. Data for some of our patients in the study group support this hypothesis: Patients who were positive for >1 autoantibody at postpartum had a higher probability to keep their autoimmune antibodies when reexamined. Persistent (resting) autoimmunity could lead to the later onset of autoimmune disease, as known for diabetes. However, the 2 subjects who remained positive for TGAB and TPAB during follow-up did not show any symptom of thyroid disorder or any other disease.

Glucose intolerance was only partly associated with signs of autoimmunity in our study. Only 8 (Table 2) of 22 patients with glucose intolerance at postpartum also had autoimmune antibodies. Thyroid antibodies as an additional sign for autoimmunity were associated with glucose intolerance in 28% of patients in the study group. Of the 2 patients who continued to exhibit GADA during the follow-up, 1 had IGT. It is possible that, in this case, HELLP syndrome caused persistent glucose intolerance, leading to type 1 diabetes or latent autoimmune diabetes in adults (25) later on. This hypothesis is supported by the previous description of the development of type 1 diabetes in a patient with HELLP syndrome (14). Only 1 of the 2 patients with diabetes diagnosed postpartum had type 1 diabetes but was possibly already antibody negative. In our study, 31% of the HELLP patients had glucose intolerance at postpartum, whereas in the control group only 6.6% had glucose intolerance. According to data published by the American Diabetes Association (26), glucose intolerance is found in ~4% of normal pregnancies. No data about the frequency of glucose intolerance in gestosis or HELLP syndrome patients have been reported so far. Of our HELLP patients, 5.7% had persisting glucose intolerance during follow-up. This finding is consistent with the assumption that HELLP syndrome is a risk factor for the development of glucose intolerance. Furthermore, our HELLP patients, with normal OGTTs at postpartum, had significantly higher glucose values at fasting, 1 h, and 2 h compared with control subjects. Besides immunological causes, we may speculate that insulin resistance could play an additional role in augmenting the development of IGT and diabetes in these patients. This association is already known for patients with gestational diabetes who are at increased risk for the development of type 2 diabetes when obesity and other factors that promote insulin resistance are present.

In conclusion, our study, for the first time, describes a high frequency of autoimmune phenomena and glucose intolerance in patients with HELLP syndrome. Although part of these alterations are only transient, we believe that this observation is of clinical relevance. If our results are confirmed by long-term follow-up and additional studies, it may become advisable to repeatedly test patients with a history of HELLP syndrome for the presence of glucose intolerance. If the development of

autoimmune disease will also be shown, it may become recommendable to test such patients for autoimmune antibodies, too.

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