Effect of cholesterol-lowering agents on soluble epidermal growth factor receptor level in type 2 diabetes and hypercholesterolemia

Jun Choul Lee, MD, PhD, Kyong Hye Joung, MD, PhD, Ji Min Kim, MD, PhD, Seon Mee Kang, MD, PhD, Hyun Jin Kim, MD, PhD, Bon Jeong Ku, MD, PhD

Abstract
Soluble epidermal growth factor receptor (sEGFR) levels are elevated in patients with type 2 diabetes mellitus (T2DM) and positively correlate with blood glucose and cholesterol levels. However, how cholesterol-lowering treatment in patients with T2DM affects the sEGFR level is unknown. Therefore, we investigated the change of serum sEGFR after cholesterol-lowering treatment in type 2 diabetic patients with hypercholesterolemia. This study is a non-randomized, prospective observational study. A total of 115 patients were treated in either the rosuvastatin monotherapy group (R group, 5 mg/day, n = 59) or the rosuvastatin/ezetimibe combination therapy group (RE group, 5 mg/10mg/day, n = 56) for 12 weeks. We measured serum levels of lipids and sEGFR using an ELISA kit before and after 12 weeks of treatment in each group. The low-density lipoprotein cholesterol (LDL-C) level was significantly reduced (from 130.27 ± 27.09 to 76.24 ± 26.82 mg/dL; P < .001) after 12 weeks of treatment and more so in the RE group than in the R group (from 131.68 ± 28.72 to 87.13 ± 27.04 mg/dL; P < .001 in the R group; from 128.78 ± 25.58 to 64.75 ± 21.52 mg/dL; P < .001 in the RE group; R vs RE group, P < .001). The sEGFR level was significantly decreased after 12 weeks of treatment (from 50.34 ± 13.31 to 45.75 ± 11.54 ng/mL; P = .007). The RE group only showed a significant reduction in the sEGFR level after treatment (from 50.94 ± 12.10 to 44.80 ± 11.36 ng/mL; P = .007). Moreover, the sEGFR level was significantly reduced only when the LDL-C level was significantly reduced (from 50.46 ± 10.66 to 46.24 ± 11.86 ng/mL; P = .043). The serum sEGFR level was significantly reduced by cholesterol-lowering treatment with rosuvastatin alone or rosuvastatin/ezetimibe. We suggested that sEGFR may play a significant role in insulin resistance (IR) and inflammation, which are central pathophysiological mechanisms. We confirmed the possibility of using sEGFR as a biomarker to predict a good response to lipid-lowering treatment in type 2 diabetes patients with hypercholesterolemia.

Keywords: diabetes, ezetimibe, inflammation, LDL cholesterol, soluble EGFR, statin

1. Introduction
The epidermal growth factor receptor (EGFR; also known as ErbB1 and HER1) gene is a transmembrane tyrosine kinase receptor belonging to the ErbB family that is expressed in various cells of epithelial, mesenchymal, and neuronal origin.[1] The EGFR is activated by binding to various ligands, activating various downstream pathways. Typical pathways include the Ras/Raf/mitogen-activated protein kinase pathway, which modulates cell proliferation and survival, and the phosphatidylinositol 3-kinase/Akt pathway, which regulates cell growth, apoptosis resistance, invasion, and migration.[2,3] In addition, EGFR plays roles in tumor cell survival, proliferation, metastasis, and tumor angiogenesis.[4]

The authors have no conflicts of interest to disclose.

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

This research protocol was approved by the Institutional Review Board of Chungnam National University Hospital (No. CNUH-2018-10-030-001) and the RE group than in the R group (from 131.68 ± 28.72 to 87.13 ± 27.04 mg/dL; P < .001 in the R group; from 128.78 ± 25.58 to 64.75 ± 21.52 mg/dL; P < .001 in the RE group; R vs RE group, P < .001). The sEGFR level was significantly decreased after 12 weeks of treatment (from 50.34 ± 13.31 to 45.75 ± 11.54 ng/mL; P = .007). The RE group only showed a significant reduction in the sEGFR level after treatment (from 50.94 ± 12.10 to 44.80 ± 11.36 ng/mL; P = .007). Moreover, the sEGFR level was significantly reduced only when the LDL-C level was significantly reduced (from 50.46 ± 10.66 to 46.24 ± 11.86 ng/mL; P = .043). The serum sEGFR level was significantly reduced by cholesterol-lowering treatment with rosuvastatin alone or rosuvastatin/ezetimibe. We suggested that sEGFR may play a significant role in insulin resistance (IR) and inflammation, which are central pathophysiological mechanisms. We confirmed the possibility of using sEGFR as a biomarker to predict a good response to lipid-lowering treatment in type 2 diabetes patients with hypercholesterolemia.

Abbreviations: ALT = alanine transaminase, AST = aspartate transaminase, BUN = blood urea nitrogen, EGFR = epidermal growth factor receptor, HbA1c = glycosylated hemoglobin, HDL-C = high-density lipoprotein cholesterol, IR = insulin resistance, LDL-C = low-density lipoprotein cholesterol, R group = rosuvastatin monotherapy group, RE group = rosuvastatin/ezetimibe combination therapy group, sEGFR = soluble epidermal growth factor receptor, T2DM = type 2 diabetes mellitus, TC = total cholesterol, TG = triglyceride.

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1. Introduction
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Soluble epidermal growth factor receptor (sEGFR) present in serum is formed via an alternative splicing or ectodomain shedding process. This soluble isoform contains only the extracellular domain of full-length EGFR. sEGFR is detected not only in normal tissues and serum but also in cancer cells. Therefore, recent studies have suggested that sEGFR is a useful circulating biochemical marker in cancer patients.[9]

Recent studies have also shown the relevance of sEGFR in metabolic diseases such as type 2 diabetes mellitus (T2DM) and metabolic syndrome, demonstrating higher sEGFR levels in patients with T2DM compared with healthy controls. In addition, univariate analyses confirmed that the sEGFR level in patients newly diagnosed with T2DM is correlated with glycosylated hemoglobin (HbA1c) and fasting and 2-hour postprandial serum glucose levels, suggesting its potential as a diagnostic marker for T2DM.[10] Furthermore, Kyohara et al confirmed that sEGFR is correlated with insulin resistance (IR) in db/db mice, which exhibit severe obesity and hyperglycemia. The serum sEGFR level in patients with T2DM increases in proportion to the levels of total cholesterol (TC), high-density lipoprotein cholesterol (HDL-C), and the homeostatic model assessment of insulin resistance (HOMA-IR).[11] The serum sEGFR level in humans might also be correlated with fasting blood glucose, fasting serum insulin, and HbA1c levels. In addition, higher TC, low-density lipoprotein cholesterol (LDL-C), and triglyceride (TG) levels, often observed in T2DM or metabolic syndrome patients, are positively correlated with the serum sEGFR level.

However, it remains unknown how cholesterol-lowering treatment in patients with T2DM accompanied by hypercholesterolemia affects the sEGFR level. Therefore, we measured serum sEGFR levels before and after rosuvastatin plus ezetimibe combination therapy in patients with T2DM and hypercholesterolemia.

2. Methods

2.1. Participants

This study is a non-randomized, prospective observational study. We enrolled patients from the outpatient clinic of the Division of Endocrinology and Metabolism of Chungnam National University Hospital (Daejeon, South Korea) between October 2017 and August 2020. The inclusion criteria were as follows: age ≥ 18 years, diagnosis of T2DM, LDL-C level ≥ 100 mg/dL, no alcohol or drug abuse, no allergy to statins or ezetimibe, absence of any clinical sign of infection or inflammation, normal serum levels of aspartate transaminase (AST), alanine transaminase (ALT), and creatine kinase, and no pregnancy. Participants with malignant neoplasms or uncontrolled hypothyroidism were excluded, and were those taking lipid-lowering therapy or hormone replacement therapy after menopause because these drugs can affect lipid metabolism. A total of 120 patients were enrolled. They were treated in either the rosuvastatin monotherapy group (R group, 5mg/day, n = 60) or the rosuvastatin/ezetimibe combination therapy group (RE group, 5mg/10mg/day, n = 60) for 12 weeks to lower LDL cholesterol below 100 mg/dL by the guidelines in use in Korea.[12] Two treatment regimens were applied alternately in the order in which they agreed to participate in this study. One subject in the R group and 4 subjects in the RE group dropped out due to withdrawal (n = 3) or loss of follow-up (n = 2). Finally, a total of 115 patients were evaluated for the study. We measured serum lipid and sEGFR levels before and after 12 weeks of treatment. Our experimental protocol was performed according to the Declaration of Helsinki, and the institutional review board of Chungnam National University Hospital approved the study protocol (No. CNUH 2018-10-030-001). All patients provided written informed consent.

2.2. Biochemical parameter measurements

All biochemical parameters were concurrently measured using standard methods at the Chungnam National University Hospital using TBA 200FR (Toshiba Medical Systems, Tokyo, Japan), Cobas e801 (Roche Diagnostics, Mannheim, Germany), Coulter DxH900 (Beckman Coulter, Miami, FL), and Variant II Turbo (Bio-Rad, Hercules, CA). Blood samples were collected using ethylenediaminetetraacetic acid tubes and serum clot activator tubes (Becton, Dickinson and Company, Franklin Lakes, NJ) in the morning after an overnight fast (≥ 8 hours). The lipid profile (HDL-C, LDL-C, TC, and TG) was measured using a blood chemistry analyzer (Hitachi 747; Hitachi, Tokyo, Japan). Glycosylated hemoglobin level was measured by high-performance liquid chromatography (Bio-Rad Laboratories, Hercules, CA).

2.3. Measurement of the serum sEGFR level

We measured serum EGFR levels using a commercial enzyme-linked immunosorbent assay kit (Catalog No. ab193764; Abcam, Cambridge, UK) according to the manufacturer's instructions. The intra- and interassay coefficients of variation were 3.7% to 5.5% and 9.4% to 10.0%, respectively.

2.4. Statistical analyses

Continuous variables are expressed as the means ± standard deviation, and between-group differences in the continuous variables were assessed using Student t test or the Kruskal–Wallis test. Categorical variables are expressed as counts and percentages, and between-group differences in the categorical variables were assessed using the chi-squared test. To determine the factors influencing the sEGFR and lipid levels before and after treatment, the paired t test was performed on all participants, and the association was analyzed separately in both groups. P < .05 was considered statistically significant. Statistical analyses were conducted using SPSS Statistics, version 25.0 (SPSS Inc., Chicago, IL).

3. Results

3.1. Clinical characteristics of the patients before and after treatment with rosuvastatin alone or rosuvastatin/ezetimibe

The average age of the 115 participants in the study was 55.80 ± 12.13 years. The average duration of T2DM was 5.09 ± 6.28 years, and 47.8% (n = 55) of the patients were men. We examined the changes in the baseline characteristics of the entire patient cohort after 12 weeks of treatment (Table 1). TC, TG, and LDL-C levels decreased significantly after rosuvastatin monotherapy or rosuvastatin/ezetimibe combination therapy for 12 weeks, whereas the HDL-C level increased significantly. All patients maintained T2DM treatment during the 12-week study period, and both the average HbA1c and fasting serum glucose levels decreased after treatment, but not significantly. After treatment, AST, ALT, and total bilirubin levels increased significantly. However, none of the patients experienced clinical adverse events associated with rosuvastatin or ezetimibe use, nor did liver enzyme levels increase above the normal range. No patient dropped out of the study because of these adverse events. Hemoglobin, platelet, serum calcium, and phosphate levels changed significantly from before to after treatment; however, these changes were within the normal range and were clinically insignificant.

3.2. Baseline characteristics of the patients in the R and RE groups

The baseline clinical characteristics were compared between the R and RE groups (Table 2). The average ages were
Table 1
Clinical characteristics of the study subjects before and after treatment with rosuvastatin and rosuvastatin/ezetimibe.

| Before | After |
|--------|-------|
| (n = 115) | (n = 115) | P value |
| WBC (×10^3/μL) | 6.82 ± 1.72 | 6.85 ± 1.82 | .626 |
| Hb (g/dL) | 14.10 ± 1.52 | 14.36 ± 1.38 | .005 |
| Platelet (×10^3/μL) | 241.09 ± 57.48 | 223.06 ± 54.60 | < .001 |
| HbA1c (%) | 6.92 ± 1.17 | 6.80 ± 0.97 | .361 |
| Fasting glucose (mg/dL) | 154.36 ± 65.71 | 145.57 ± 37.85 | .118 |
| BUN (mg/dL) | 15.06 ± 4.00 | 14.73 ± 4.16 | .122 |
| Creatinine (mg/dL) | 0.85 ± 1.17 | 0.72 ± 0.19 | .346 |
| Calcium (mg/dL) | 8.94 ± 0.35 | 8.93 ± 0.38 | .031 |
| Phosphate (mg/dL) | 3.43 ± 0.54 | 3.53 ± 0.51 | .003 |
| ALT (IU/L) | 21.88 ± 10.58 | 28.41 ± 15.90 | .015 |
| AST (IU/L) | 21.88 ± 6.80 | 24.69 ± 8.32 | .01 |
| Total bilirubin (mg/dL) | 0.83 ± 0.25 | 0.98 ± 0.28 | .005 |
| TC (mg/dL) | 210.14 ± 25.03 | 210.31 ± 25.10 | .870 |
| Calcium (mg/dL) | 8.94 ± 0.35 | 8.93 ± 0.38 | .031 |
| HbA1c (%) | 7.05 ± 1.32 | 6.78 ± 0.98 | .219 |
| BUN (mg/dL) | 15.06 ± 4.00 | 14.73 ± 4.16 | .122 |
| DM duration (years) | 5.30 ± 5.94 | 4.86 ± 6.69 | .743 |
| Male (%) | 27 (45.8%) | 28 (50.0%) |
| Age (years) | 57.41 ± 12.00 | 54.11 ± 12.13 | .145 |

Data are presented as means ± standard deviation. The Student t test calculated P value.

Table 2
Baseline characteristics of the patients in the rosuvastatin monotherapy and rosuvastatin/ezetimibe combination therapy groups.

| R group | RE group |
|---------|----------|
| (n = 59) | (n = 56) | P value |
| Age (years) | 57.41 ± 12.00 | 54.11 ± 12.13 | .145 |
| Male (%) | 27 (45.8%) | 28 (50.0%) |
| DM duration (years) | 5.30 ± 5.94 | 4.86 ± 6.69 | .743 |
| HbA1c (%) | 7.05 ± 1.32 | 6.78 ± 0.98 | .219 |
| Fasting glucose (mg/dL) | 152.95 ± 60.69 | 155.86 ± 71.46 | .636 |
| TC (mg/dL) | 210.14 ± 25.03 | 210.31 ± 25.10 | .870 |
| TG (mg/dL) | 158.16 ± 69.68 | 177.50 ± 95.88 | .337 |
| LDL-C (mg/dL) | 131.68 ± 28.72 | 128.78 ± 25.58 | .021 |
| eSEGR (ng/mL) | 49.78 ± 14.44 | 50.94 ± 12.10 | .644 |

Data are presented as means ± SDs or number (%). The Student t test calculated P value.

57.41 ± 12.00 years (range, 31–78 years) in the R group and 54.11 ± 12.13 years (range, 18–78 years) in the RE group (P = .145). There were 27 men (45.8%) in the R group and 28 men (50.0%) in the RE group. The average T2DM duration was 5.30 ± 5.94 years in the R group and 4.86 ± 6.69 years in the RE group (P = .743). There were no differences between the groups in several biochemical indicators, including the HbA1c level, fasting glucose level, and lipid profile. Baseline eSEGR levels were 49.78 ± 14.44 ng/mL in the R group and 50.94 ± 12.10 years (range, 18–78 years) in the RE group (P = .644).

3.3. Changes in the LDL-c level after treatment with rosuvastatin alone or rosuvastatin/ezetimibe

In all patients, the LDL-C level was significantly reduced after 12 weeks of treatment (from 130.27 ± 27.09 to 76.24 ± 26.82 mg/dL; P < .001) (Fig. 1A). LDL-C level was significantly reduced after treatment and was lower in the RE group than in the R group (from 131.68 ± 28.72 to 87.13 ± 27.04 mg/dL, P < .001 in the R group; from 128.78 ± 25.58 to 64.75 ± 21.32 mg/dL, P < .001 in the RE group; R vs RE groups, P < .001) (Fig. 1B).

3.4. Changes in the sSEGR level after treatment with rosuvastatin alone or rosuvastatin/ezetimibe

The serum sSEGR level was significantly decreased after 12 weeks of treatment in all patients (from 50.34 ± 13.31 to 45.75 ± 11.54 ng/mL; P = .007) (Fig. 2A). The RE group showed a significant reduction in the sSEGR level after treatment (from 50.94 ± 12.10 to 44.80 ± 11.36 ng/mL; P = .007) (Fig. 2B), whereas the R group showed no difference (from 49.78 ± 14.44 to 46.64 ± 11.74 ng/mL; P = .224).

3.5. Changes in the serum sSEGR level according to the baseline LDL-C level and degree of LDL-c change after treatment

When we assessed differences in the baseline sSEGR level with respect to the LDL-C level before treatment. We calculated the median serum LDL-C level before treatment and used it to divide the patients into high and low baseline LDL-C level groups. There was a tendency for a slightly higher sSEGR level in the patients with a higher baseline LDL-C level, but the difference was not significant (52.14 ± 14.65 vs 48.08 ± 11.13 ng/mL in the high vs low baseline LDL-C groups; P = .104) (Fig. 3A). The median degree of change in the LDL-C level from before to after treatment in all patients was determined and used to divide the patients according to a significant decrease vs slight decrease (high and low LDL-C difference group). sSEGR level was significantly reduced after treatment only in the high LDL-C difference group (from 50.46 ± 10.66 to 46.24 ± 11.86 ng/mL; P = .043 in the high LDL-C difference group; from 50.22 ± 15.65 to 45.24 ± 11.29 ng/mL; P = .071 in the low LDL-C difference group) (Fig. 3B).

4. Discussion

T2DM, obesity, and CVD share a metabolic milieu characterized by IR and chronic subacute inflammation.[13] TNF-α, IL-6, and other proinflammatory cytokines appear to participate in the induction and maintenance of the subacute inflammatory state associated with obesity-induced IR.[14] MCP-1 and other chemokines are essential to recruiting macrophages to adipose tissue.[15] These cytokines and chemokines activate intracellular pathways that promote the development of IR and T2DM.[16] And other various humoral factors act through interorgan crosstalk during the progression of T2DM or dyslipidemia caused by IR.[17] Circulating factors used as IR-related markers have been identified. In addition, these biomarkers could reflect therapeutic efficacy more accurately in patients with early-stage T2DM or dyslipidemia based on IR and inflammation.

Activation of the EGFR signaling pathway is linked to regulating several cellular responses, including inflammatory processes. Previously, we induced the excessive activation of EGFR by hepatic MIG6 knockout in mice and found that they have abnormalities related to glucose and cholesterol metabolism, characterized by marked increases in LDL cholesterol, fasting glucose and HOMA-IR.[18,19] Therefore, we suggested that activation of EGFR may be deeply involved in the progression of diabetes and dyslipidemia. And through other recent studies, it is well known that EGFR activation is involved in the inflammatory process in chronic kidney disease[20] and a number of skin disorders, such as psoriasis or atopic dermatitis.[21]
In diabetes, sEGFR is produced in metabolic organs such as the liver and adipose tissue. The increased EGFR expression in these organs directly affects their serum concentration. Its expression level is regulated in parallel with its regulation of insulin levels under diabetic conditions. [11] Various studies have found that the serum sEGFR concentration increases with increasing TC, LDL-C, and TG levels, the lipid abnormalities commonly observed in T2DM and metabolic syndrome. [11,22] In previous studies, we measured sEGFR levels in patients with newly diagnosed T2DM; [10] however, here, we measured sEGFR levels in patients who had T2DM for over 5 years on average. sEGFR level was elevated in patients with high HbA1c and fasting blood glucose levels before treatment (data not shown). Furthermore, although not statistically significant, the sEGFR level tended to be high in patients with high LDL-C levels before treatment (Fig. 3A). Therefore, based on these results, we determined that sEGFR could be an additional diagnostic tool to assess the degree of inflammation or IR in metabolic diseases such as diabetes.

LDL-C in the body is maintained by de novo synthesis in the liver and by its intestinal absorption from the diet and bile; these 2 primary sources are the main therapeutic targets for hypercholesterolemia. Statin treatment targeting cholesterol synthesis and ezetimibe treatment targeting cholesterol absorption can be used in combination to lower LDL-C more effectively. In this study, we found that the LDL-C level was significantly reduced after 12 weeks of treatment with rosuvastatin alone or rosuvastatin/ezetimibe (Fig. 1A). And we found a significant reduction in the sEGFR level after rosuvastatin and ezetimibe treatment in patients with T2DM and hypercholesterolemia (Fig. 2A).
addition to targeting cholesterol synthesis to lower the blood level of LDL-C, statin has an anti-inflammatory effect.\[23\] And ezetimibe has an additional positive effect on metabolic disorders. Hiramitsu et al showed that ezetimibe therapy significantly reduces the level of high-sensitivity C-reactive protein, a representative marker of atherosclerotic diseases.\[24\] So we could conclude that these cholesterol-lowering drugs decrease sEGFR concentration by inhibiting inflammatory processes in diabetes patients with hypercholesterolemia.

Then we assessed the change in the sEGFR level in the R and RE groups. sEGFR level was significantly decreased after treatment only in the RE group (Fig. 2B); therefore, the reduced sEGFR level may be due to a significant reduction in the LDL-C level, considering that the LDL-C level was reduced significantly more by the rosuvastatin/ezetimibe combination therapy than by rosuvastatin monotherapy after 12 weeks of treatment (Fig. 1B). We assessed the effect of the LDL-C reduction on the changes in the sEGFR level. We found no change in the sEGFR level in patients with a slight reduction in the LDL-C level after treatment. However, in the patients with a considerable reduction in the LDL-C level, the sEGFR level was significantly reduced (Fig. 3B). Therefore, we confirmed the possibility of using sEGFR as a biomarker that can predict a good response to lipid-lowering treatment in type 2 diabetes patients with hypercholesterolemia.

Lastly, it is worth paying attention to the diabetes-related pleiotropic effect of ezetimibe concerning the fact that a significant change in the blood concentration of sEGFR was observed only in the RE group. Studies in Zucker fatty rats, a model of obesity, have also shown that ezetimibe therapy improves IR.\[23\] Furthermore, clinical studies have shown that ezetimibe therapy can significantly reduce adiponectin, an obesity marker associated with lipid and glucose metabolism, as well as fasting insulin and HbA1c levels.\[25\] In another study, low-dose pravastatin and ezetimibe combination significantly improved IR compared with high doses of pravastatin monotherapy.\[27\] Even if the effect of improving IR and inflammation is not associated with an LDL-C reduction, it is clear that ezetimibe can improve them when combined with statins.\[24\] In this study, apart from the LDL-C reduction effect, ezetimibe likely reduced the sEGFR level via IR and inflammation improvements.

This study had several limitations. First, since all patients continued to control their blood glucose levels using antidiabetic drugs, it is impossible to rule out the possibility that these drugs affected the sEGFR level changes. Second, we did not measure HOMA-IR values or the levels of several inflammatory markers as objective indicators of an improvement in IR. Finally, this study was conducted at a single institution with a small number of patients, decreasing the statistical power. So further studies are needed to identify the precise mechanisms underlying the changes in the sEGFR level. Nevertheless, to the best of our knowledge, this is the first study to show that the level of sEGFR can change after treating metabolic diseases, suggesting its use as a biochemical marker in serum.

5. Conclusion
We found that the serum sEGFR level was significantly reduced by cholesterol-lowering treatment in patients with type 2 diabetes and hypercholesterolemia. We suggested that sEGFR plays a significant role in IR and inflammation, which are central pathophysiological mechanisms in T2DM with hypercholesterolemia.

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