DOI: 10.4274/gulhane.galenos.2021.40427
Gulhane Med J 2022;64:217-21

Changes in neutrophil-to-lymphocyte ratio following treatment with dapagliflozin in patients with type 2 diabetes mellitus

Süleyman Baş[1], Funda Müşerref Türkmen[2]

[1]*University of Health Sciences Türkiye, Sancaktepe Sehit Prof. Dr. Ilhan Varank Training and Research Hospital, Clinic of Internal Medicine, Istanbul, Türkiye*

[2]*University of Health Sciences Türkiye, Haydarpasa Numune Training and Research Hospital, Clinic of Internal Medicine, Istanbul, Türkiye*

Date submitted:
05.07.2021
Date accepted:
21.12.2021
Online publication date:
08.09.2022

Corresponding Author:
Süleyman Baş, M.D., University of Health Sciences Türkiye, Sancaktepe Sehit Prof. Dr. Ilhan Varank Training and Research Hospital, Clinic of Internal Medicine, Istanbul, Türkiye
suleymanbas.2012@gmail.com

ORCID:
orcid.org/0000-0002-5883-445X

Keywords: Dapagliflozin, neutrophil-to-lymphocyte ratio, sodium-glucose cotransporter-2 inhibitor, type 2 diabetes mellitus

ABSTRACT

Aims: Dapagliflozin, a sodium-glucose cotransporter-2 inhibitor, is indicated for glycemic control in type 2 diabetes mellitus (T2DM). In this study, we investigated the changes in neutrophil-to-lymphocyte ratio (NLR), a marker of inflammation, in patients with T2DM after the initiation of dapagliflozin.

Methods: This retrospective study included patients (aged 18 to 75 years) with T2DM who were prescribed dapagliflozin 10 mg once daily additional to their existing diabetic treatment. Patients with a history of chronic liver disease, chronic renal failure, infection, inflammatory disease, and the use of drugs affecting bone marrow were included. The duration of treatment was set at 12 weeks.

Results: The study included 98 patients with a mean age of 54.3±8.0 years, with a female predominance of 61.2%. At 12th week, there was a statistically significant decrease in fasting glucose (199.6 mg/dL vs. 164.3 mg/dL, p<0.001), glycated hemoglobin (HbA1c) (8.92% vs. 8.01%, p<0.001), leukocyte count (7.79 10^3/mm^3 vs. 8.36 10^3/mm^3, p=0.018) and neutrophil count (4.44 10^3/mm^3 vs. 4.84 10^3/mm^3, p=0.027). Lymphocyte count (2.56 10^3/mm^3 vs. 2.72 10^3/mm^3, p=0.150) and NLR (1.86 vs. 1.89, p=0.758) also showed some increase but the difference was not statistically significant.

Conclusions: This study showed significant increases in leukocyte and neutrophil counts in T2DM patients taking dapagliflozin, but lymphocyte count and NLR remained unaltered.

Introduction

Diabetes mellitus (DM) is a chronic disease characterized by the inability of the body to use insulin effectively or the inability of the pancreas to produce sufficient insulin (1).

Chronic inflammation plays an important role in the development and progression of DM and its complications. Some studies show that there is an increase in the levels of inflammatory cytokines such as C-reactive protein, interleukin-1 (IL-1), interleukin-6 (IL-6), and tumor necrosis factor-alpha (TNF-α) in patients with diabetes (2,3).

The ratio of neutrophil count to lymphocyte count (neutrophil to lymphocyte ratio, NLR) is accepted as an indicator of systemic inflammation. NLR has been increased in inflammatory conditions such as hepatosteatosis, malignant conditions, thyroiditis, cardiac conditions, and inflammatory bowel disease (4-10). NLR is considered a marker of inflammation in diabetes and its microvascular and macrovascular complications (11-13).

Additionally, studies have described the relationship between NLR and glycemic control in patients with diabetes. Studies have shown that mean NLR values are higher in patients with poor glycemic control compared to patients with good glycemic control (14,15).

Dapagliflozin is a sodium-glucose cotransporter-2 (SGLT-2) inhibitor used for treating type 2 DM (T2DM). SGLT-2 inhibitors cause SGLT-2 inhibition in the renal proximal tubules, reducing the glucose reabsorption from the kidney and increasing glucose excretion through the urinary tract (1). Since they act independently of insulin, they can be used in any stage of diabetes (1).

Dapagliflozin has positive effects on the heart and kidneys (16-18). While it shows these effects, its effects on inflammation are not exactly known. In our study, we investigated the effect of dapagliflozin on inflammation by looking at NLR levels before and after dapagliflozin.

Methods

This retrospective study included patients with T2DM who were started treatment with dapagliflozin 10 mg once daily at the Internal Medicine Outpatient Clinics of the University of Health Sciences Türkiye, Haydarpaşa Numune Training and Research Hospital between July 2017 through December 2018. The main enrollment criterion was the use of dapagliflozin in addition to the existing treatment for at least 12 weeks. Additional inclusion criteria were age between 18 and 75 years and estimated glomerular filtration >60 mL/min/1.73 m^2. Patients with chronic liver disease and/or transaminase levels above 5 times the upper limit of normal, hematological diseases, on drugs affecting the bone marrow, splenic disease, active infectious diseases, acute or chronic inflammatory diseases, and malignancy were excluded. Patients on glucagon-like peptide 1 analogs were excluded. Patients who were on statins for more than 24 weeks at the time of dapagliflozin treatment were included.

The demographic characteristics, chronic diseases, fasting glucose, glycated hemoglobin (HbA1c), blood urea nitrogen (BUN), creatinine, hematocrit, thrombocyte count, leukocyte count, neutrophil count, and lymphocyte count were recorded. NLR was calculated by dividing the absolute neutrophil count by the absolute lymphocyte count from the complete blood count. The same variables were recorded in the next visit, at least after 12 weeks of dapagliflozin treatment.

The study was approved by the University of Health Sciences Türkiye, Haydarpasa Numune Training and Research Hospital (22.04.2019/5386). All human studies were conducted under the rules of the Declaration of Helsinki.

Statistical Analysis

Statistical analysis for the Statistical Package for the Social Sciences Statistics 23 (IBM Corp., Armonk, NY, USA) software was used to perform statistical calculations. The normality of the distribution was evaluated by the Kolmogorov-Smirnov test. Descriptive statistical methods, including percentage and mean±standard deviation or median (interquartile range), were used to display the basic characteristics. Associations between glucose, HbA1c and NLR variables were assessed using Pearson's correlation analysis. A paired t-test was used to compare biochemical and hematological parameters before and after dapagliflozin in patients with T2DM. The statistical significance level was set as $p<0.05$.

Results

A total of 98 patients, including 60 females (61.2%) and 38 males (38.8%), were included in the study. The mean age of the patients was 54.3 years. The median diabetes duration of the patients was 7 years (Table 1). Thirty-eight patients had hypertension, 41 patients had dyslipidemia, 10 patients had coronary artery disease and 2 patients had cerebrovascular disease. Four patients had diabetic retinopathy and 22 patients had diabetic neuropathy.

Mean basal glucose was 199.6±73.2 mg/dL, HbA1c was 8.9±1.7%, BUN was 13.9±3.5 mg/dL, creatinine was 0.79±0.08 mg/dL, leukocyte count was 7.79±1.35 $10^3/mm^3$, neutrophil count was 4.44±1.08 $10^3/mm^3$, lymphocyte count was 2.56±0.67 $10^3/mm^3$, and NLR was 1.86±0.76 (Table 2).

A statistically significant positive correlation was observed between glucose and NLR values (r=0.206, p=0.043). There was no significant correlation between HbA1c and NLR values (r=0.140, p=0.170) (Table 3).

A significant decrease was observed in glucose and HbA1c levels after dapagliflozin treatment (p<0.001, p<0.001). Although there was an increase in BUN and creatinine levels, this change was not statistically significant (p=0.07, p=0.614). A statistically significant increase was detected in leukocyte and neutrophil levels (p=0.018, p=0.027). A statistically insignificant

Table 1. Demographic characteristics and comorbid diseases (n=98)

Age (years), mean±SD (range)	54.3±8.0 (31-69)
Gender, n (%)	
Female	60 (61.2)
Male	38 (38.8)
Diabetes duration (years), median (IQR)	7 (4.75-9)
Diabetic retinopathy, n (%)	4 (4.1)
Diabetic neuropathy, n (%)	22 (22.4)
Hypertension, n (%)	38 (38.8)
Dyslipidemia, n (%)	41 (41.8)
Coronary artery disease, n (%)	10 (10.2)
Cerebrovascular disease, n (%)	2 (2)

SD: Standard deviation, IQR: Interquartile range

increase was detected in lymphocyte and NLR levels (p=0.150, p=0.758). Significant increases were detected in HbA1c and hematocrit levels (p=0.028, p=0.028). A nonsignificant increase was documented in the platelet level (p=0.573).

Discussion

In this study, we observed no significant change in the NLR values in patients with diabetes after dapagliflozin treatment. On the other hand, there was a significant relationship between glucose and NLR, and significant increases in leukocyte, neutrophil, HbA1c, and hematocrit levels. A significant decrease was also observed in glucose and HbA1c levels. There was no significant change in lymphocyte or platelet levels.

DM is not only a metabolic disease but also inflammatory mechanisms play an important role from its development to its complications (19). NLR has been used as an inflammatory marker (20). Concerning potential correlations between NLR, glucose, and HbA1c, different results can be encountered in the literature. Buyukkaya et al. (21) showed a positive association between serum glucose and NLR elevation. Although Verdoia et al. (8) reported a statistically significant positive correlation between NLR and serum glucose, they did not observe any relationship between NLR and HbA1c. However, Sefil et al. (22) showed a positive association between NLR and HbA1c levels. Kim et al. (23) also reported a positive correlation between NLR and fasting plasma glucose in T2DM patients, but they could not find a relationship between NLR and HA1c. In our study, a positive statistically significant correlation was noted between NLR and glucose in T2DM patients, and no significant relationship was found between NLR and HbA1c. NLR values may differ according to age (24). The different ages of the patients included in the studies may have been effective in obtaining the different results between NLR and HbA1c in the studies. The patient ages in our study were similar to those in the study by Kim et al. (23).

Several authors have shown the effects of SGLT-2 inhibitors on inflammation. Maayah et al. (25) demonstrated the positive effects of empagliflozin on lipopolysaccharide-induced septic shock in mice. It has also been shown that empagliflozin reduces systemic and renal inflammation that leads to the development of sepsis-related kidney injury. Heerspink et al. (26) showed that canagliflozin decreases the levels of TNF receptor-1, IL-6, matrix metalloproteinase-7, and fibronectin-1 in patients with T2DM. Kohlmorgen et al. (27) reported that dapagliflozin did not affect leukocyte, neutrophil, and lymphocyte counts. Although there was a significant increase in leukocyte and neutrophil levels in our study, there was no change in NLR values. Wortmann et al. (28) reported that empagliflozin increased neutrophil levels and improved neutrophil functions by decreasing the intracellular 1,5-anhydroglucitol-6-phosphate (1,5-AG-6-P) level in patients with glycogen storage disease type 1b (GSD1b) (28). Resaz et al. (29) showed improvement in neutrophil functions by reducing the accumulation of 1,5-AG-6-P in myeloid cells in their study

Table 2. Biochemical and hematological parameters before and after dapagliflozin in patients with T2DM

	Before dapagliflozin treatment	After dapagliflozin treatment	p
Glucose (mg/dL), mean±SD	199.63±73.24	164.30±53.07	<0.001*
HbA1c (%), mean±SD	8.92±1.70	8.01±1.63	<0.001*
BUN (mg/dL), mean±SD	13.90±3.50	14.50±3.46	0.07
Creatinine (mg/dL), mean±SD	0.79±0.08	0.80±0.14	0.614
Leukocyte (10^3/mm^3), mean±SD	7.79±1.35	8.36±1.92	0.018*
Neutrophil (10^3/mm^3), mean±SD	4.44±1.08	4.84±1.37	0.027*
Lymphocyte (10^3/mm^3), mean±SD	2.56±0.67	2.72±0.81	0.150
NLR, mean±SD	1.86±0.76	1.89±0.68	0.758
HbA1c (g/dL), mean±SD	13.12±1.76	13.69±1.83	0.028*
Hematocrit (%), mean±SD	40.05±4.94	41.64±5.10	0.028*
Platelet (10^3/mm^3), mean±SD	267.24±66.72	261.64±71.53	0.573

*p<0.05.
NLR: Neutrophil lymphocyte ratio, HbA1c: Glycated hemoglobin, BUN: Blood urea nitrogen, T2DM: Type 2 diatebes mellitus

Table 3. Pearson correlation analysis between glucose and HbA1c and NLR before dapagliflozin treatment in patients with T2DM

	NLR	
	r*	p
Glucose	0.206	0.043**
HbA1c	0.140	0.170

*r: Pearson's correlation coefficient, **p<0.05.
HbA1c: Glycated hemoglobin, NLR: Neutrophil lymphocyte ratio, T2DM: Type 2 diatebes mellitus

on induced GSD1b mouse models. In our study, the increase in neutrophil count may be due to the effect of dapagliflozin on the intracellular 1,5-AG-6-P level.

Hematocrit levels increase during SGLT-2 inhibitor therapy (30). Since this increase in hematocrit during SGLT-2 inhibitor treatment was accompanied by an increase in the BUN creatinine ratio, it was interpreted that it could be related to hemoconcentration due to the diuretic effect of the SGLT-2 inhibitor (31). Recent studies show that this increase in hematocrit is not solely due to hemoconcentration. It is estimated that erythropoiesis increases with treatment with SGLT-2 inhibitor treatment (32). In patients with diabetes, the erythropoietin level increases after the initiation of SGLT-2 inhibitors (33). After an increase in the number of reticulocytes, HbA1c and hematocrit levels increase (33). Ghanim et al. (34) showed that another mechanism may be effective in the increase in hematocrit values. They suggested that the increase in hematocrit values was related to increased erythropoiesis due to decreased hepcidin levels after dapagliflozin treatment (34).

In our study, a statistically significant increase was observed in HbA1c and hematocrit levels before and after dapagliflozin treatment. This increase in HbA1c and hematocrit values was not accompanied by a significant increase in BUN and creatinine values, which we can count as hemoconcentration indicators. As mentioned in the literature (32,34), this increase may be due to the improvement of possible tubulointerstitial damage in our patients, as well as to the increase in erythropoiesis due to the decrease in hepcidin level.

SGLT-2 inhibitors are recommended in the ADA 2021 diabetes guideline as the second choice after lifestyle modification and metformin therapy in patients with atherosclerotic cardiovascular disease and chronic kidney disease (35). Ferrannini et al. (36) reported that dapagliflozin monotherapy provided a statistically significant reduction of 0.66% in HbA1c after 24 weeks of treatment compared to placebo. In our study, a statistically significant decrease of 0.91% in mean HbA1c was detected after 12 weeks of treatment with dapagliflozin 10 mg once daily.

This study has some limitations. This study was a retrospective study. The most important limitation is the lack of detailed information about the use of drugs and/or the diseases affecting the biochemical and hematological parameters of the patients. The absence of a control group is also among the limitations of the study.

Conclusion

This study showed that treatment of patients with T2DM with dapagliflozin beyond 12 weeks was not associated with a significant change in NLR, despite alterations observed in some other hematological indices. Changes in hematological parameters in patients using dapagliflozin suggest that leukocytes, neutrophils, lymphocytes, and NLR cannot be used as inflammatory markers in these patients.

Ethics

Ethics Committee Approval: Approval for this study was given by the University of Health Sciences Türkiye, Haydarpasa Numune Training and Research Hospital (protocol no: 5386, date: 22.04.2019).

Informed Consent: Retrospective study.

Peer-review: Externally and internally peer-reviewed.

Authorship Contributions

Concept: S.B, F.M.T., Design: S.B, F.M.T., Data Collection or Processing: S.B., Analysis or Interpretation: S.B., Literature Search: S.B, F.M.T., Writing: S.B.

Conflict of Interest: No conflict of interest was declared by the authors.

Financial Disclosure: The authors declared that this study received no financial support.

References

1. The Society of Endocrinology and Metabolism of Turkey (SEMT) Diabetes Mellitus Working Group. Clinical Practice Guideline for Diagnosis, Treatment and Follow-up of Diabetes Mellitus and Its Complications 2019. 12th ed. (English Version). Ankara: Bayt;2019.
2. de Rooij SR, Nijpels G, Nilsson PM, et al. Low-grade chronic inflammation in the relationship between insulin sensitivity and cardiovascular disease (RISC) population: associations with insulin resistance and cardiometabolic risk profile. Diabetes Care. 2009;32:1295-1301.
3. Garcia C, Feve B, Ferré P, et al. Diabetes and inflammation: fundamental aspects and clinical implications. Diabetes Metab. 2010;36:327-338.
4. Aktas G, Taslamacioglu Duman TT, Kurtkulagi Ö, et al. Liver steatosis is associated both with platelet distribution width, neutrophil/lymphocyte and monocyte/lymphocyte ratios. Prim Health Care. 2020;10:1-4.
5. Sit M, Aktas G, Erkol H, et al. Neutrophil to lymphocyte ratio is useful in differentiation of malign and benign thyroid nodules. P R Health Sci J. 2019;38:60-63.
6. Aktas G, Sit M, Dikbas O, et al. Elevated neutrophil-to-lymphocyte ratio in the diagnosis of Hashimoto's thyroiditis. Rev Assoc Med Bras (1992). 2017;63:1065-1068.
7. Paquissi FC. The predictive role of inflammatory biomarkers in atrial fibrillation as seen through neutrophil-lymphocyte ratio mirror. J Biomark. 2016;2016:8160393.
8. Verdoia M, Schaffer A, Barbieri L, et al. Impact of diabetes on neutrophil-to-lymphocyte ratio and its relationship to coronary artery disease. Diabetes Metab. 2015;41:304-311.
9. Azab B, Chainani V, Shah N, McGinn JT. Neutrophil-lymphocyte ratio as a predictor of major adverse cardiac

events among diabetic population: a 4-year follow-up study. Angiology. 2013;64:456-465.

10. Posul E, Yilmaz B, Aktas G, Kurt M. Does neutrophil-to-lymphocyte ratio predict active ulcerative colitis? Wien Klin Wochenschr. 2015;127:262-265.
11. Shiny A, Bibin YS, Shanthirani CS, et al. Association of neutrophil-lymphocyte ratio with glucose intolerance: an indicator of systemic inflammation in patients with type 2 diabetes. Diabetes Technol Ther. 2014;16:524-530.
12. Akbas EM, Demirtas L, Ozcicek A, et al. Association of epicardial adipose tissue, neutrophil-to-lymphocyte ratio and platelet-to-lymphocyte ratio with diabetic nephropathy. Int J Clin Exp Med. 2014;7:1794-1801.
13. Moursy EY, Megallaa MH, Mouftah RF, Ahmed SM. Relationship between neutrophil lymphocyte ratio and microvascular complications in Egyptian patients with type 2 diabetes. Am J Intern Med. 2015;3:250-255.
14. Hussain M , Babar MZM , Akhtar L , Hussain MS. Neutrophil lymphocyte ratio (NLR): A well assessment tool of glycemic control in type 2 diabetic patients. Pak J Med Sci. 2017;33:1366-1370.
15. Devamash GN, Parvathi M, Madhumathi R, Leela R. Study of neutrophil lymphocyte ratio in patients with type 2 diabetes mellitus and its correlation with glycemic control. Int J Adv Med. 2019;6:1637-1641.
16. Tanajak P, Sa-Nguanmoo P, Sivasinprasasn S, et al. Cardioprotection of dapagliflozin and vildagliptin in rats with cardiac ischemia-reperfusion injury. J Endocrinol. 2018;236:69-84.
17. Wang FZ, Wei WB, Li X, et al. The cardioprotective effect of the sodium-glucose cotransporter 2 inhibitor dapagliflozin in rats with isoproterenol-induced cardiomyopathy. Am J Transl Res. 2021;13:10950-10961.
18. Thomas MC. Renal effects of dapagliflozin in patients with type 2 diabetes. Ther Adv Endocrinol Metab. 2014;5:53-61.
19. Öztürk ZA, Kuyumcu ME, Yesil Y, et al. Is there a link between neutrophil-lymphocyte ratio and microvascular complications in geriatric diabetic patients? J Endocrinol Invest. 2013;36:593-599.
20. Liu CC, Ko HJ, Liu WS, et al. Neutrophil-to-lymphocyte ratio as a predictive marker of metabolic syndrome. Medicine (Baltimore). 2019;98:e17537.
21. Buyukkaya E, Karakas MF, Karakas E, et al. Correlation of neutrophil to lymphocyte ratio with the presence and severity of metabolic syndrome. Clin Appl Thromb Hemost. 2014;20:159-163.
22. Sefil F, Ulutas KT, Dokuyucu R, et al. Investigation of neutrophil lymphocyte ratio and blood glucose regulation in patients with type 2 diabetes mellitus. J Int Med Res. 2014;42:581-588.
23. Kim JK, Lee AY, Kang JH, Yu BY, Kim SJ. Association of fasting glucose level with neutrophil-lymphocyte ratio compared to leukocyte count and serum c-reactive protein. Korean J Fam Med. 2018;39:42-50.
24. Li J, Chen Q, Luo X, et al. Neutrophil-to-lymphocyte ratio positively correlates to age in healthy population. J Clin Lab Anal. 2015;29:437-443.
25. Maayah ZH, Ferdaoussi M, Takahara S, Soni S, Dyck JRB. Empagliflozin suppresses inflammation and protects against acute septic renal injury. Inflammopharmacology. 2021;29:269-279.
26. Heerspink HJL, Perco P, Mulder S, et al. Canagliflozin reduces inflammation and fibrosis biomarkers: a potential mechanism of action for beneficial effects of SGLT2 inhibitors in diabetic kidney disease. Diabetologia. 2019;62:1154-1166.
27. Kohlmorgen C, Gerfer S, Feldmann K, et al. Dapagliflozin reduces thrombin generation and platelet activation: implications for cardiovascular risk reduction in type 2 diabetes mellitus. Diabetologia. 2021;64:1834-1849.
28. Wortmann SB, Van Hove JLK, Derks TGJ, et al. Treating neutropenia and neutrophil dysfunction in glycogen storage disease type Ib with an SGLT2 inhibitor. Blood. 2020;136:1033-1043.
29. Resaz R, Raggi F, Segalerba D, et al. The SGLT2-inhibitor dapagliflozin improves neutropenia and neutrophil dysfunction in a mouse model of the inherited metabolic disorder GSDIb. Mol Genet Metab Rep. 2021;29:100813.
30. Sano M, Goto S. Possible mechanism of hematocrit elevation by sodium glucose cotransporter 2 inhibitors and associated beneficial renal and cardiovascular effects. Circulation. 2019;139:1985-1987.
31. Aberle J, Menzen M, Schmid SM, et al. Dapagliflozin effects on haematocrit, red blood cell count and reticulocytes in insulin-treated patients with type 2 diabetes. Sci Rep. 2020;10:22396.
32. Sano M, Takei M, Shiraishi Y, Suzuki Y. Increased hematocrit during sodium-glucose cotransporter 2 inhibitor therapy indicates recovery of tubulointerstitial function in diabetic kidneys. J Clin Med Res. 2016;8:844-847.
33. Lambers Heerspink HJ, de Zeeuw D, Wie L, Leslie B, List J. Dapagliflozin a glucose regulating drug with diuretic properties in subjects with type 2 diabetes. Diabetes Obes Metab. 2013;15:853-862.
34. Ghanim H, Abuaysheh S, Hejna J, et al. Dapagliflozin suppresses hepcidin and increases erythropoiesis. J Clin Endocrinol Metab. 2020;105:dgaa057.
35. American Diabetes Association. 9. Pharmacologic approaches to glycemic treatment: standards of medical care in diabetes-2021. Diabetes Care. 2021;44(Suppl 1):111-1124.
36. Ferrannini E, Ramos SJ, Salsali A, Tang W, List JF. Dapagliflozin monotherapy in type 2 diabetic patients with inadequate glycemic control by diet and exercise: a randomized, double-blind, placebo-controlled, phase 3 trial. Diabetes Care. 2010;33:2217-2224.