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Original article

Long-term follow-up observations confirm the stability of hemoglobin F levels in patients with hemoglobinopathies receiving metformin therapy for type 2 diabetes

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Abstract. *Background and aim:* There is limited real-world evidence on how metformin use may affect fetal hemoglobin (HbF) levels in patients with hemoglobinopathies. This study describes long-term changes in HbF levels in patients with sickle cell anemia (SCA) and thalassemia major who continuously received metformin at a standard dose for type 2 diabetes. *Methods:* Adult patients taking metformin for at least six months and had at least three different time points of HbF assessments at three months apart were included. Pharmacy refill records were used to evaluate adherence to metformin. Data were collected before metformin therapy, and during routine follow up visits. Comparisons were performed using the Wilcoxon matched pairs test. *Results:* Over a period of ten years, five patients with SCA (71%) and two (29%) with thalassemia major were enrolled. Metformin was administered orally at doses ranging from 1000 to 2000 mg per day, either alone or concurrently with hydroxyurea. Among the participants, four patients (57%) underwent allogeneic bone marrow transplantation after 12 to 60 months of initiation of metformin therapy. When compared to baseline levels, HbF levels did not exhibit a significant change following one to eight years of metformin therapy alone or in combination with hydroxyurea (P=0.58). *Conclusions:* Collectively, our findings indicate that the clinical utilization of metformin at standard doses, either alone or added to hydroxyurea does not have any additional effect on HbF levels among patients with hemoglobinopathies even after several years of therapy. (www.actabiomedica.it)

Key words: metformin, hydroxyurea, hemoglobin f, hemoglobinopathies, type 2 diabetes mellitus

Introduction

Metformin is recommended as a first line treatment for patients with type 2 diabetes, overweight and obese nondiabetic individuals (1). It offers potential advantages for patients with sickle cell disease (SCD) by inducing *in-vitro* fetal hemoglobin (HbF), a key genetic modifier of clinical severity of the disease (2). In addition, metformin usage in SCD patients with diabetes was associated with significant reductions in the

most common SCD-related complications, regardless of the study design, and other confounders (3). However, the lack of data on HbF levels in this cross-sectional retrospective cohort study limits its use as a HbF booster.

To date, only two exploratory observational studies have investigated the effects of metformin on HbF levels in clinical practice, and none of these demonstrated a significant increase in HbF levels following a standard dose of metformin in patients with or without SCD (4, 5). Notably, a clinical trial evaluating the impact of metformin on HbF levels in patients with SCD is currently underway, but the results are still pending (ClinicalTrials.gov Identifier: NCT02981329). However, a recent small prospective interventional study in non-transfusion dependent thalassemia patients reported a limited benefit of metformin in enhancing HbF levels (6). To contribute to the understanding of the role of metformin on the reactivation of HbF expression *in-vivo*, the present retrospective study aimed to capture the real-world experience regarding the use of metformin and its effectiveness in inducing HbF changes in an unselected population of patients with SCA and thalassemia.

Patients and Methods

Study design and subjects

This was a retrospective longitudinal observational study conducted at the SQU Hospital (SQUH) from 1st January 2007 to 31 December 2022. Data were collected from patients attending the outpatient clinics and the pharmacy records at SQUH, which provides a free of cost medical care to all Omanis. The study included national Omani patients aged over 15 years, taking metformin for at least six months and having at least three HbF assessments at three months intervals. The medication history, including dietary supplements and multivitamins of the enrolled patients was carefully evaluated.

Data collection and follow-up visits

The data extraction process involved utilizing a case report form designed to retrieve information from the electronic database of Sultan Qaboos University Hospital (SQUH). Socio-demographic characteristics, baseline and follow-up HbF levels, treatment details, and outcomes were abstracted for each patient. Information regarding metformin prescriptions, doses, and durations was collected during the follow-up visits. Two independent investigators carefully reviewed all completed data collection forms to ensure clarity and consistency. Enrolled patients were monitored from the initial HbF reading before commencing metformin therapy until three months following the last HbF assessment, resulting in varying durations of individual follow-up. Of note, HbF levels were measured in the absence of blood transfusions for at least four consecutive weeks.

Pharmacy refill adherence

The pharmacy refill adherence was measured using electronic pharmacy records. It was defined as the collection of the prescribed metformin refills from the SQUH pharmacy. The period between refills was defined as the days between the last prescription of metformin and the next collected refill. Adherence over the previous 3 months was defined as [(pills dispensed/pills prescribed per day) / days between refills) ×100], as previously reported by Grossberg *et al.* (7). Calculated refill adherence rate values above 100% for patients who refilled earlier than scheduled were rounded to 100 %.

Outcome measures

The primary outcome was the evaluation of HbF levels, which were determined by the cation-exchange high-performance liquid chromatography (HPLC; Bio-Rad VARIANT, Bio-Rad Laboratories, Hercules, CA, USA). HbF levels were expressed as a percentage of the total hemoglobin variants. The complete blood count was assessed using the automated CELL-DYN™ Sapphire (Abbott Laboratories, Chicago, Illinois, USA) analyzer, and glycated hemoglobin A1c (HbA1c) using Integra 400, as previously reported (5).

Statistical analysis

Statistical analysis was conducted using the Graph Pad PRISM 5.0 software (Graph Pad Software

Inc., San Diego, CA, USA). A significance level of $p < 0.05$ was chosen to determine statistically significant differences. Quantitative data were summarized using mean, standard deviation, and range. The Wilcoxon matched pairs test was used to evaluate the differences among study variables.

Results

Over a period of ten years, seven patients with hemoglobinopathies receiving metformin to treat type-2 diabetes were identified and enrolled (Table 1). Among of them five patients (71%) were diagnosed with sickle cell anemia (SCA), while two patients (29%) had thalassemia major.

Before initiation of metformin therapy, all patients exhibited a severe clinical phenotype necessitating regular blood transfusions, and frequent admissions to the outpatient clinics. At metformin initiation, the total hemoglobin levels ranged from 7.6 g/dL to 9.5 g/dL with a mean value of 8.7 g/dL. Subsequently, four patients (57%) underwent an allogeneic bone marrow transplantation (BMT) after 12 to 60 months of initiation of metformin. Of note, metformin therapy was not suspended during BMT and blood transfusions were used if clinically required only. Metformin was administered orally at maintenance doses ranging from 1000 to 2000 mg/day (Table 1). The average duration of metformin treatment was four years, with a range of one to nine years and a median of four years. Among the patients with SCA, two (28.5%) received metformin and hydroxyurea concurrently for at least five years, while three (43%) received additional insulin treatment. Using adherence threshold of ≥ 90%, all patients (100%) were classified as adherent by the pharmacy refill method. Prior to metformin initiation, HbA1c readings were available in five (71%) patients. The levels of HbA1c tend to decrease over time from 6.1 ± 1 % (range: 4.9 - 7.4%) at 12-16 months compared to levels prior to metformin therapy 7.3 ± 2% (range: 6-9.3%). Figure 1A and 1B show changes of HbF levels over time in SCA patients after starting metformin alone (43%), and metformin with hydroxyurea (28.5%), respectively. Both treatment approaches failed to demonstrate an increase in HbF levels among SCA patients even after one to eight years of therapy. Notably, HbF levels remained unchanged in all transplanted patients with SCA following BMT. Similarly, in thalassemia patients (28.5%) metformin alone was not associated with an increase in HbF levels (Figure 1C).

Figure 2 illustrates the comparison of HbF levels before the initiation of metformin therapy and one year after treatment in the study population before BMT. No statistically significant difference in HbF levels was found between baseline and one year after imitation of metformin therapy (P=0.57).

Discussion

Over the last decade, our understanding of the pathophysiology of β-hemoglobinopathies has remarkably improved. As a result, considerable advancements have been made in developing treatment options for SCD. However, economic constraints have hindered the widespread utilization of these new therapies in many countries with a high prevalence of SCD (8). In this context, metformin has emerged as a promising option for patients with β-hemoglobinopathies owing to its well-established safety profile and relatively affordable cost. Indeed, recent studies have opened the floodgates on metformin in SCD (2, 3). The real-world experience studies like ours complement clinical trials to make meaningful observations regarding changes in HbF levels in patients with hemoglobinopathies receiving metformin alone or in combination with hydroxyurea at standard doses. The present study provides conclusive evidence that metformin alone is insufficient to elevate HbF levels, even after prolonged and continuous therapy over a median period of four years. These findings confirm and expand upon the results of a recent small clinical trial conducted in non-transfusion-dependent thalassemia patients, which demonstrated a limited role of metformin in inducing HbF expression *in-vivo* (6). Moreover, our results align with those reported by Han *et al.* (4) who found that although metformin led to a slight, non-significant increase in HbF levels in three of five patients with sickle β0-thalassemia, its overall effect on HbF levels was minimal. Similarly, Boulassel *et al.* (5)

Table 1. Baseline characteristics of study population at initiation of metformin therapy for type 2 diabetes

Case	Gen	Sex	Age at T2D/ Met*	Age at Hu*	Age at BMT	T2D Hist	BMI	Com	Met Dose*	HU Use*	HU Dose*	Hb*	WBC*	PLT*	SF*	AST/ ALT*
1	S/S	M	18	-	19	No	28	Yes	1000	No	-	9.7	7.9	349	1354	46/103
2	S/S	M	21	-	22	No	21	Yes	1500	No	-	9.1	7.7	352	3423	39/85
3	S/S	F	46	-	-	No	18	Yes	1500	No	-	9.9	12.7	162	20425	71/175
4	S/S	M	21	20	26	Yes	29	Yes	2000	Yes	14	8.8	10.7	168	140	13/23
5	S/S	M	32	22	-	No	24	Yes	2000	Yes	7	11	7.8	153	170	124/409
6	ß-thal major	F	16	-	17	No	24	Yes	1000	No	-	10.1	4.4	194	700	18/29
7	HbE/ ß-thal	M	57	-	-	No	44	Yes	2000	No	-	10.5	9.5	207	157	35/69

Abbreviations: Gen: Genotype; S/S: sickle cell anemia; ß-thal major: ß-thalassemia major; T2D: type 2 diabetes; Met: metformin; Hu: hydroxyurea; BMT: Allogeneic bone marrow transplantation; Hist: Clinical history of T2D; BMI: Body mass index (Kg/m^2); Com: Comorbidities; Hb: hemoglobin (g/dL); WBC: White blood count (x10^9/L); PLT: platelet count (x10^9/L); SF: Serum ferritin (ng/mL); AST: aspartate aminotransferase (Units/L); ALT: alanine aminotransferase (Units/L); *at initiation of metformin.

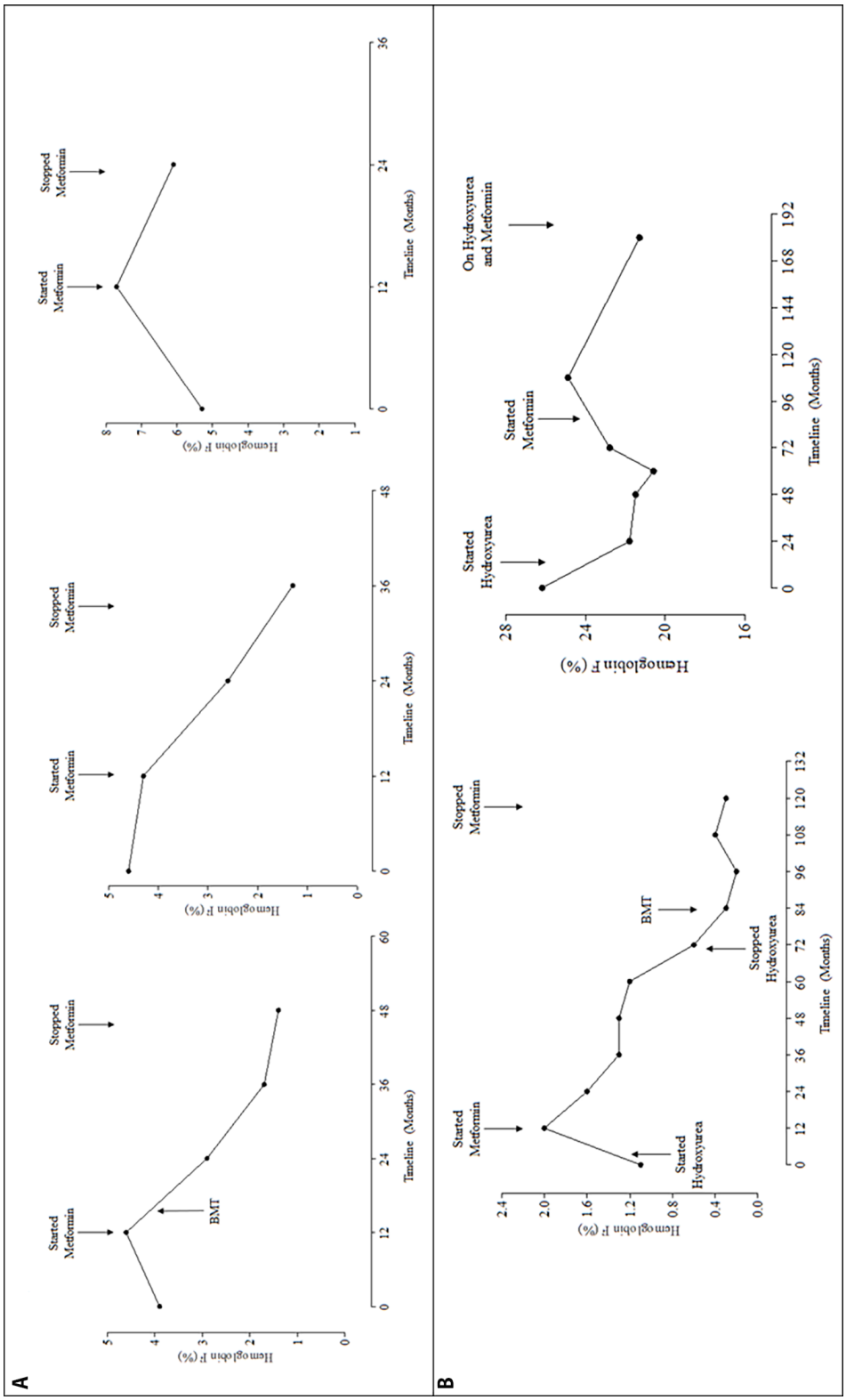

Figure 1. Changes in hemoglobin F levels over time in patients with sickle cell anaemia (A) receiving metformin alone, (B) metformin added to hydroxyurea, and (C) in patients with thalassemia major taking metformin alone.

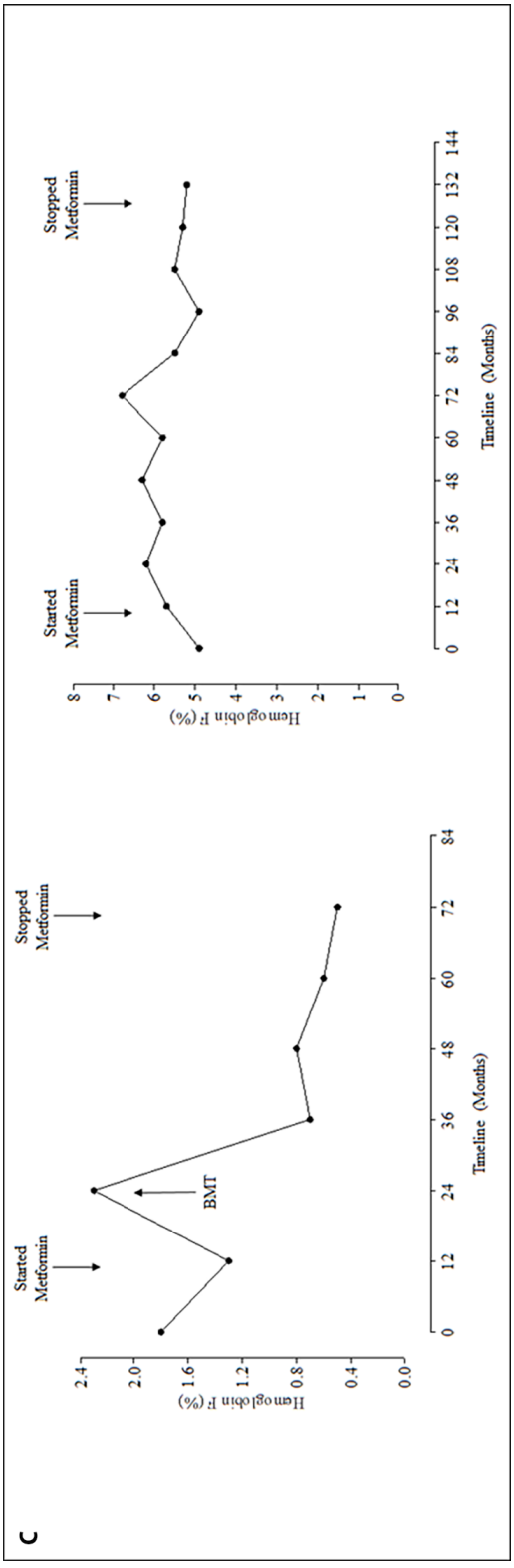

Figure 1. (*Continued*)

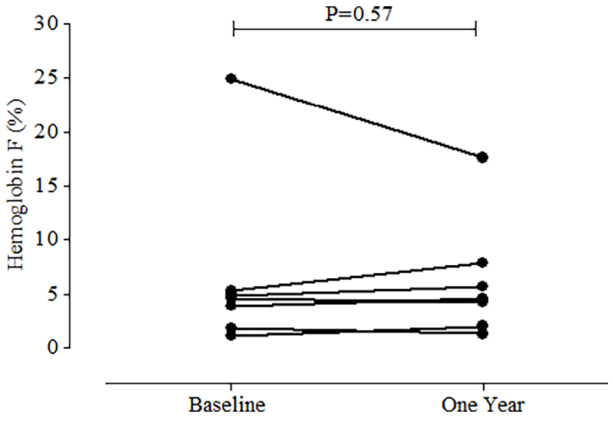

Figure 2. Changes in hemoglobin F levels at metformin initiation and one year after therapy in the study population before allogeneic bone marrow transplantation.

in an observational study, reported that metformin alone, administered at doses of 1000-2000 mg/day, did not increase HbF levels in patients with type 2 diabetes or polycystic ovary syndrome. These collective observations further support our findings, demonstrating the absence of significant changes in HbF levels following one year of metformin therapy. Interestingly, an *in vitro* study revealed a significant increase of approximately 46% in HbF levels when hydroxyurea was administered concurrently with metformin in hematopoietic stem cell (HSC) cultures obtained from individuals with normal hemoglobin and patients with SCD (2). However, these *in vitro* findings correlate poorly with the *in vivo* results observed in the present study and other recent investigations (4-6). Specifically, our study failed to demonstrate any advantage of combining metformin with hydroxyurea in terms of HbF levels, even after an extended period of therapy. Similarly, the co-administration of metformin and hydroxyurea for six months did not yield statistically significant differences in clinical and laboratory parameters, including HbF levels, in patients with non-transfusion-dependent thalassemia (6). Likewise, an observational study reported no significant increase in HbF levels when a combination of metformin and hydroxyurea was administered to patients with sickle β^0-thalassemia (4). The unexpectedly weak correlation between *in vivo* outcomes and *in vitro* results may be partially attributed to the inherent simplicity of cell cultures, which are relatively straightforward systems compared to the intricacy of living tissues. Another explanation is that in some patients receiving hydroxyurea with a good clinical response, no detectable rise in HbF has been noticed, indirectly reflecting the lack of association between HbF and hydroxyurea therapy (9). Finally, it could be the variation in genetic elements that influence HbF production or differences in drug bioavailability and metabolism among the patients (10). From a mechanistic standpoint, metformin may exhibit a more substantial effect on stressed HSC that are already pushed into a proliferative cycle, thereby facilitating the reactivation of silenced fetal gammaglobin genes and leading to the production of red cells containing HbF (2). Consequently, it would be expected that transplanted patients with hemoglobinopathies who are taking metformin would experience a substantial increase in HbF levels as these patients undergo both erythropoietic proliferative stress and the stress associated with bone marrow transplantation for several months, followed by a return to normal hematopoiesis. However, our findings appear to contradict this observation, as we observed no noticeable increase in HbF levels before or after bone marrow transplantation in SCD patients receiving metformin alone or in combination with hydroxyurea. Even in severe chronic bone marrow stress, metformin does not exert potent genetic modification effects on HbF levels *in vivo*, regardless of the administered doses and the timing of follow-up visits.

Our study has several potential limitations that should be considered. First, we did not directly measure the circulating levels of metformin and instead relied on an indirect assessment of drug adherence. However, the pharmacy refill procedure is a reliable and validated method for measuring adherence behaviour in clinical settings (7). Moreover, although HbA1c may not be the most accurate marker for diabetes in SCD patients (11), the observed trend towards a decrease in HbA1c levels from baseline suggests that patients were indirectly adhering to metformin therapy. It is important to point out that alternative methods monitoring glycemic control in patients with hemoglobinopathies have been proposed to overcome HbA1c limitations. Among these methods, measurement of fructosamine levels, fasting and non-fasting self-monitoring blood

glucose levels, as well as continuous glucose monitoring devices (12). Second, it is possible that a more extended treatment period is necessary to observe the effects of metformin therapy on HbF levels. However, our findings demonstrate that long-term therapy for more than five years did not lead to an increase in HbF levels despite the varying duration of metformin therapy among patients. Therefore, it is unlikely that a longer duration of treatment would explain the lack of an increase in HbF levels. Third, the effects of metformin were altered by other concurrently taken drugs or products. However, this explanation seems unlikely, as medication that could affect the effectiveness of metformin was well-documented in all patients, although the presence of non-prescribed medicines cannot be entirely ruled out. Fourth, our study's limited number of patients may have reduced the power to detect an increase in HbF levels following metformin therapy. However, it is important to note that this was a real-world observational study conducted over a ten-year period, where data sources and diagnoses were based on physician reporting, and the outcomes were compared within each individual, minimizing the potential impact of the sample size. Finally, it is also plausible that suppression of erythropoiesis by means of blood transfusions caused a halt in HbF formation. However, early studies have shown that HbF production reactivates as the patients retuned to the anemic state (13). In conclusion, our study provides compelling evidence that metformin therapy, whether used alone or combined with hydroxyurea, does not increase HbF levels even after long-term treatment. Additional combined strategies using more potent fetal gamma-globin gene modifiers might be required to sufficiently induce HbF expression *in-vivo*, which could potentially lead to reduce hemolysis thereby easing symptoms in patients with hemoglobinopathies.

Ethic Approval: This study was performed in accordance with the Declaration of Helsinki and was approved by the Research Ethics Committee (REC) of the Sultan Qaboos University (SQU), Sultanate of Oman (January 2022, MERC#2115). In addition, approval was obtained from the hospital management before accessing to the medical records of the patients. Patient consent was waived by the REC due to the retrospective chart review design of this study.

Conflict of Interest: Each author declares that he or she has no commercial associations (e.g. consultancies, stock ownership, equity interest, patent/licensing arrangement etc.) that might pose a conflict of interest in connection with the submitted article

Authors Contribution: MMH, MMH and HA participated in study deign, data collection, processing and analysis; HK, IG, RNQ, VP, ZQ, EA, and YW performed primary diagnosis, clinical assessments and classification of participants, and MRB designed, performed, analyzed data, and wrote the manuscript. All co-authors critically revised and approved the manuscript.

Declaration on the Use of AI: None.

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References

1. Sanchez-Rangel E, Inzucchi SE. Metformin: clinical use in type 2 diabetes. Diabetologia. 2017; 60 (9):1586–93. doi: 10.1007/s00125-017-4336-x.
2. Zhang Y, Paikari A, Sumazin P, et al. Metformin induces FOXO3-dependent fetal hemoglobin production in human primary erythroid cells. Blood. 2018; 132 (3):321–33. doi: 10.1182/blood-2017-11-814335.
3. Badawy SM, Payne AB. Association between clinical outcomes and metformin use in adults with sickle cell disease and diabetes mellitus. Blood Adv. 2019;3 (21):3297–306. doi: 10.1182/bloodadvances.2019000838.
4. Han J, Saraf SL, Molokie RE, Gordeuk VR. Use of metformin in patients with sickle cell disease. Am J Hematol. 2019; 94 (1):E13–5. doi: 10.1002/ajh.25313.
5. Boulassel MR, El-Hussain AAI, Hassan MM, et al. Stability of fetal hemoglobin levels in patients receiving metformin therapy. Haematologica. 2018; 103 (10):e440–2. doi: 10.3324/haematol.2018.
6. ElTagui M, El-Ghamrawy M, AlDeeb SK, Nassim MS. Metformin as a fetal hemoglobin inducer in non-transfusion dependent thalassemia patients. Indian J Hematol Blood Transfus. 2024; 40 (1):68-73. doi: 10.1007 /s12288-023-01662-1.
7. Grossberg R, Zhang Y, Gross R. A time-to-prescription-refill measure of antiretroviral adherence predicted changes in viral load in HIV. J Clin Epidemiol. 2004; 57(10): 1107–10. doi: 10.1016/j.jclinepi.2004.04.002.

8. Esoh K, Wonkam-Tingang E, Wonkam A. Sickle cell disease in sub-Saharan Africa: transferable strategies for prevention and care. Lancet Haematol. 2021; 8(10):e744–5. doi: 10.1016/S2352-3026(21)00191-5.
9. Charache S. Mechanism of action of hydroxyurea in the management of sickle cell anemia in adults. Semin Hematol. 1997; 34 (3 Suppl 3):15-21.
10. Steinberg MH, Lu Z-H, Barton FB, et al. Fetal hemoglobin in sickle cell anemia: determinants of response to hydroxyurea. Blood. 1997; 89:1078-1088.
11. McLean A, Wright F, deJong N, et al. Hemoglobin A_{1c} and fructosamine correlate in a patient with sickle cell disease and diabetes on chronic transfusion therapy. Pediatr Blood Cancer. 2020; 67(9):e28499. doi: 10.1002/pbc.28499.
12. Obeagu EI, Obeagu GU. Management of diabetes mellitus patients with sickle cell anemia: Challenges and therapeutic approaches. Medicine (Baltimore). 2024; 103(17):e37941. doi: 10.1097/MD.0000000000037941
13. Gabuzda TG, Gardner FH. Regulation of fetal and adult hemoglobin formation in patients with sickle cell disease transfused to normal hematocrits. Blood. 1967; 29(1):126-33.

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