OPEN ACCESS

Saudi Journal of Medical and Pharmaceutical Sciences
Abbreviated Key Title: Saudi J Med Pharm Sci
ISSN 2413-4929 (Print) | ISSN 2413-4910 (Online)
Scholars Middle East Publishers, Dubai, United Arab Emirates
Journal homepage: https://saudijournals.com

Original Research Article **Nephrology**

Association of FGF23 Levels and Development of Anemia in Patients with Non Dialysis Chronic Kidney Disease

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DOI: https://doi.org/10.36348/sjmps.2024.v10i09.001 | **Received:** 16.07.2024 | **Accepted:** 20.08.2024 | **Published:** 02.09.2024

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Abstract

***Background*:** Anemia is one of the common complication of Chronic Kidney Disease that intensifies in grade and severity as eGFR decline which also increases the risks for cardiovascular mortality. The development of anemia and elevated fibroblast growth factor 23 levels are the earliest changes observed in chronic kidney disease. ***Objective*:** This study aims to understand the association of FGF23 levels development of anemia in chronic kidney disease patients prior to renal replacement therapy. ***Methods*:** This is an analytical type of cross sectional study carried out among 95 respondent'1ged between 18 and 75 years with CKD stage 1-5 before dialysis, divided into two groups- Group I- comprised of 68 patients and Group 11- included 27 age and sex matched respondents not having CKD, enrolled from two te1tiary-care hospitals, namely- Sir Salimullah Medical College & Mitford Hospital and National Institute of Kidney Diseases & Urology (NIKDU). Socio-demographic status, disease profile and laboratory findings including serum iron, iron binding capacity, ferritin, transferrin saturation, calcium, phosphorus, intact parathormone, vitamin D, eGFR and FGF-23 levels were studied. ***Result*:** Mean age of the respondents was 50.1±10.74 (SD) years, mean estimated glomerular filtration rate was 35.92± 22.61 in group I and 91.66± 14.2 in group II. The mean FGF23 level in group I and II were 85.76± 207.54, 21.99± 12.12 pg/mL respectively, serum iron level was 81.61± 39.24 mcg/dL and 95.0± 32.38 mcg/dL in group I and II respectively, serum ferritin level was 225.59± 2 I 2.5 ng/mL and 157.85± 220.89 ng/ml. TIBC was 312.65± 95.83 mcg/dL and 418.85± 118.25 mcg/dL in group I and II respectively. In Group I and II, iron deficiency was found in 23% and 25%respectively when stratified according to serum ferritin level and 26.5% and 22.22% respectively, when stratified according to serum transferrin saturation level. This difference was significant among the group. Serum iron, ferritin, TIBC and TSAT were significantly associated with serum FGF23 levels. ***Conclusion*:** Disrupted iron metabolism and high FGF23 levels is commonly found in chronic kidney disease. Clinical laboratory findings have revealed the relation between FGF23 and anemia in no dialysis chronic kidney disease patients.

Keywords: Chronic Kidney Disease (CKD), Fibroblast Growth Factor 23 (FGF23), Anemia.

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INTRODUCTION

In patients with chronic kidney disease (CKD). abnormalities in mineral metabolism and the metabolic mechanism which are associated with iron deregulation is one of the commonly seen complications, and fibroblast growth factor 23 (FGF23) is a risk factor in this context. FGF23 is a bone-derived hormone that is essential for regulating vitamin D and phosphate homeostasis. In the early stages of CKD, serum FGF23 levels rise I 000-fold above normal values in an attempt to maintain normal phosphate levels' [1].

Only few human studies have suggested a possible association between FGF23 and anaemia in patients with CKD. This study was carried out to evaluate the association of FGF23 levels and development of anaemia in patients with non-dialysis chronic kidney disease.

Chronic kidney disease (CKD) is an international public health problem [2-4] With deterioration of renal CKD patients suffer from various complications such as fluid-electrolyte imbalance, acid-base abnormalities, aberrant calcium-phosphate homeostasis, and decreased red blood cell production. The prevalence of anaemia in individuals with CKD is 15.4%, and this prevalence increases from 8.4% to 53.4% with more advanced stages of CKD [4]. Renal anaemia is one of the established risk factors for cardiovascular disease "(CVD) and congestive heart failure in CKD patients [3, 4].

Clinical investigations have demonstrated significant associations between increased serum FGF23 levels. the longitudinal decline in hemoglobin levels and the overall risk incidence of anemia [5]. Furthermore. recent studies have displayed an inverse correlation with transferrin saturation levels, serum iron levels and erythropoiesis [6]. The pleiotropic actions of FGF23, along with its functional impact in renal anemia, have been qualified to direct and indirect mechanisms has been the focus of many studies [7]. FGF23 reduces the secretion of EPO from the kidney, thereby decreasing the differentiation of erythroid progenitors, such as pro-erythroblasts, to mature erythrocytes. In addition, FGF23 directly reduces the fraction of erythrocytes in the G2/M phase of their cell cycle and enhances erythrocyte apoptosis. Also, FGF23 enhances the inflammatory milieu, which in tum, promotes hepcidin excess and leads to the restriction of iron for erythropoiesis. The formation of bone, also known as osteogenesis, is essential for erythrocyte and platelet production in bone marrow [8].

Agoro *et al.*, showed a single intraperitoneal injection of FGF23 blocking peptide was sufficient to rescue renal anemia in this Nx model. These results were accompanied by the lower frequency of erythroid cell apoptosis. Collectively, these data suggest elevated serum FGF23 levels negatively regulate erythropoiesis and are negative mediator of HSC's differentiation into the erythroid linage. As for the indirect actions of FGF23. the interconnection between FGF23 and pro-inflammatory cytokines, such as in CKD, provides further insight on how FGF23 indirectly contributes to anemia. As a consequence of this enhanced inflammatory milieu, cytokines upregulate the production of hepcidin in the liver. These pathologic events foster excessive serum hepcidin levels, which in turn, promotes functional iron deficiency [8]. Till the present date. Very few clinical data on human arc available to illustrate the relationship between lower renal anemia and the serum level of FGF23 levels in non-dialysis CKD patients.

OBJECTIVE

This study aims to assess the association of FGF23 levels and Development of Anemia in Patients with Non Dialysis Chronic Kidney Disease.

METHODOLOGY

Study Design: Observational cross sectional study.

Place of Study:
Sir Salimullah Medical College & Mitford Hospital, Dhaka-I I 00.
National Institute of Kidney Diseases & Urology (NIKDU).

Period of Study: From December 2018 to November 2019.

Study Population:

Patients aged between 18 and 75 years with CKD stage 1-5 before dialysis who voluntarily provided informed consent enrolled from two tertiary-care hospitals, Sir Salimullah Medical College &-Mitford Hospital 2. National Institute of Kidney Diseases and Urology (NIKDU).

Selection Criteria:
Inclusion Criteria:
For case:

1. CKD stage 1-5 (Non-dialysis).
2. Age 18 years and above.
3. Both sexes (male and female).

For control:

1. Age and sex matched healthy control.

Exclusion criteria:

1. Chronic Inflammatory Condition.
2. Patients on steroids or NSAID.
3. Acute renal failure.
4. Chronic liver disease.
5. Malignancy.
6. Patients with hematological disorders.
7. Patients under renal replacement therapy (dialysis or kidney transplant).
8. History of internal bleeding.

Sample Size:

FGF23 level at different stages of CKD was planned to evaluate. In a previous study the standard deviation of FGF23 in optimal renal function group (CKD GI) was 28.62. The considered difference between GI and 3 group means of FGF23 was 14. By applying the formula below 64 CKD subjects in case group is needed to be able to reject the null hypothesis that the population means of two groups are equal with probability (power) 0.8. The type I error probability associated with this test of this null hypothesis is 0.05.

Sampling: Purposive sampling technique was used to select the respondents.

Research Instruments: A pretested data sheet.

Methods of Data Collection

- Data collection instrument- questionnaire, lab reports.
- Data collection technique- face to face interview.

Blood Sampling and Analysis: Blood sample was drawn from peripheral vein. Each blood sample was put into three different test tubes for hematological, biochemical investigations and allowed to clot for half an hour and then the samples was centrifuged for 15 minutes and serum was stored in ultra-deep freezer until analysis.

Data Collection Tools and Techniques: Data collection sheet consists of patient's demographics, clinical examination and investigations.

Study Procedures:

Vital signs, demographic data, co-morbid conditions and current medications taken by the patients were documented primarily, at the time of enrollment. Random blood samples were collected, centrifuged, separated into aliquots, and stored at appropriate temperature for analyses. The assessments of different parameters were conducted with standard commercial assays and automated test machine, iron profile including haemoglobin, serum iron, serum ferritin, serum TIBC, Transferrin Saturation, serum calcium, serum PO4, iPTH (pg./ml), eGFR, FGF23, Spot Urine ACR was measured. FGF23 level was measured in duplicate with a 2-site enzyme-linked immunosorbent assay that detects 2 epitopes in the carboxyl sterminal portion of FGF23. Spot urine samples were obtained to determine the urine concentration of creatinine and protein.

A questionnaire was prepared considering key variables like demographic data, clinical presentation, clinical findings, investigations were collected which was verified by the guide and the data was collected by the researcher himself.

After selection of the patient; aims, objectives and procedures of the study were explained with understandable language to the patient. Risks and benefits were also made clear to the patient. The patients were encouraged for voluntary participation and they were allowed being free to withdraw themselves from the study. Then, informed written consent was taken from each patient.

This study was performed in two steps. During the first step patients were selected according to inclusion and exclusion criteria. For Group I, Inclusion criteria were Patients of Age more than 18 years, CKD G 1,2 3a, 3b, 4, 5CKD patients and who will give consent.

Serum FGF23 was done for each patient from "Sample A" and other laboratory parameters were done from Sample B". iron profile including haemoglobin, serum iron, serum ferritin, serum TIBC, Transterrin Saturation, serum calcium, serum P04, iPTH (pg./ml), eGFR, FGF23, Spot Urine ACR were measured from "sample B" for Group I and sample c" for Group II group.

Statistical Analysis and Data Interpretation: After compilation, the data were presented in the form of tables, figures and graphs, as necessary. Statistical analysis of the results was done by using computer based statistical software. SPSS inc. Chicago, IL, USA). Quantitative data were presented as mean & standard deviation and qualitative data were presented by frequencies and percentages. Chi-square test was used for categorical variables and t test was used for quantitative variables as necessary. A P value less than 0.05 was considered as statistically significant.

RESULTS

Table I shows distribution of study subjects according to age in Group I and Group IL 36.2% study subjects were in between 4 I -50 years of age in Group I and 37% study subjects were in between 41-50 years of age in Group 11.

Table I: Distribution of study subject according to age in Group I **and Group** II **(n-95)**

Age group	Group I (n=68)	Group II (n=27)	p-value
<30	2(2.9)	1(3.7)	0.12
31 - 40	7(10.1)	5(18.5)	
41 - 50	25(36.2)	l 0(37)	
51 - 60	22(31.9)	8(29.6)	
>60	12(17.4)	3(11.1)	

Figure-1 shows distribution of the study subjects according to sex in Group I and Group II. There was no significant difference between Group I and Group II in gender distribution.

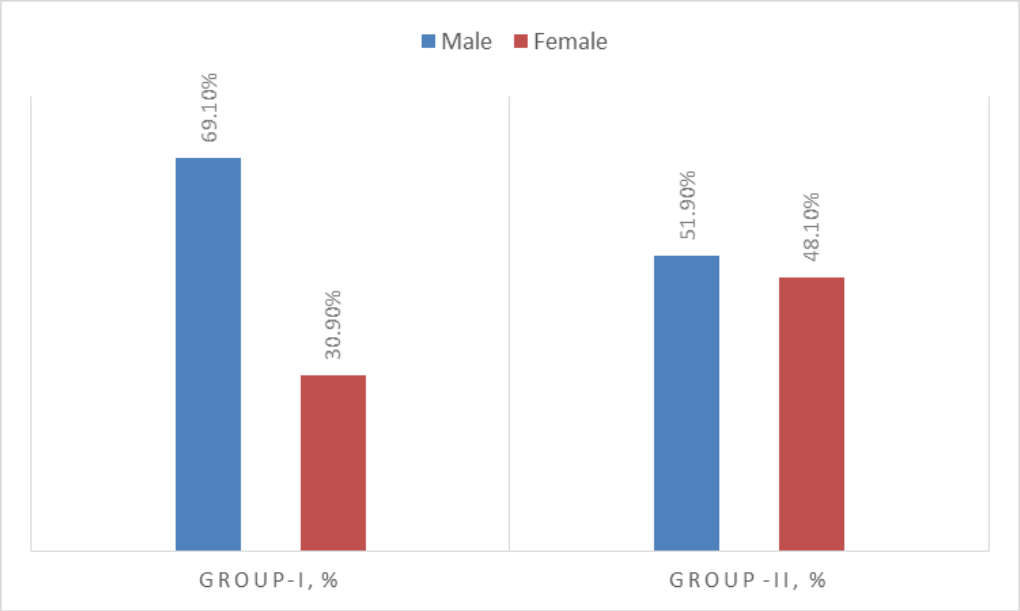

Figure 1: Gender distribution of the study group

Table II shows distribution of the study subjects according to iron deficiency anemia in terms of serum ferritin and TSAT in Group t and Group II. It has been observed that the statuses of iron deficiency anemia were significant among the groups.

Table II: Distribution of the study subjects according to iron deficiency anemia in group I and group II (n=95)

Variables	Group I (n=68)	Group II (n=27)	p-value
Serum ferritin	23(33.82)	7(25.93)	0.04
TSAT	18(26.47)	6(22.22)	0.05

Chi-square test was done to measure the level of significance.

Table-III: Hemoglobin (g/dl) distribution of the study group

Variables	Hemoglobin (g/dl)			
	β	95%CI		p-value
		Lower	Upper	
Age (per 10 years)	-0.032	- 0.094	0.030	0.32
Sex (male)	1.296	1.098	1.494	<0.001
BMI (per 1kg/m^2	0.079	0 058	0.100	<0.001
SBP (per 1 mmHg)	-0.003	-0.007	0.002	0.21
eGFR (per 1 ml/min per 1.73m^2)	0.015	0.011	0.018	<0.001
Albumin (per 1 g/dl)	0.926	0.732	1.119	<0.001
Phosphorus (per 1 mg/dl)	-0 453	-0 568	-0 338	<0001
1,25$(0H)_2$ vitamin D (per 1 pg/ml)	0.001	-0.004	0,006	0.66
Iron deficiency	-0.325	0.484	-0.166	<0.001
CRP (per I log)*	-0.019	-0.122	0.085	0.72
FGF23 (per 1 log)*	-0.067	-0.114	0.021	0.004

Table IV: Baseline biochemical parameters of the study subjects in group i and group II table

Variables	Group I (n=68)	Group II (n=27)	p-value
Serum iron	71.61± 39.24	95.0± 32.38	0.00
TIBC	312.65± 95.83	418.85± 118.25	0.00
Serum ferritin	157.85± 220.89	225.59± 212.5	0.04
TSAT	19.05±8.97	29.09± 11.5	0.02
Hemoglobin (g/dl)	11.7±1.9	12.8 ± 2.0	<0.001
Serum albumin	5.03± 0.95	5.03± 0.41	<0.001
Albumin creatinine ratio	254.6 ± 389.25	36.11 ± 95.62	<0.001

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Variables	Group I (n=68)	Group II (n=27)	p-value
Serum calcium	9.6± 1.2	8.2±0.98	0.78
Serum uric acid	5.7± 1.1	4.3± 1.4	0.15
Triglyceride level	187.1± 93.9	171.6± 98.24	0.85
Cholesterol	168.7± 75.9	183.03± 61.32	0.45
HDL	35.83± 9.2	35.66± 5.98	0.28
PTH	177.4± 162.61	17.95± 7.8	<0.001
Vitamin D	18.81± 6.97	18.25± 12.29	0.625
eGFR	35,92± 22.61	91.66± 14.2	0.023
FGF23	85.76± 207.54	21.99± 12.1	0.005
Urinary phosphate	4.25± 1.17	3.28± 0.67	0.22

Table shows serum iron, serum ferritin, TIBC, TSAT, serum calcium, serum phosphate, serum uric acid, HDL. serum PTH were found to be significantly correlated and eGFR & Hb inversely correlated the CKD stages progress.

Table V: Base line Biochemical parameters of patients according to different CKD stage

CKD stage	Stage I		Stage II		Stage III		Stage IV		Stage V		P Value
	Mean	SD	Mean	SD	Mean	SD	Mean	SD	Mean	SD	
Number	21		19		14		14				
Serum iron	89.54	38.29	103.26	38.72	86.84	38.72	74.04	24.98	72.13	36.79	0.04
Hemoglobin(g/dl)	12.30	1.8	12 .1	1.9	11.9	2.1	1 l.7	1.8	11.1	1.6	0.02
TIBC	372.46	99.07	416.90	119.23	354.84	91.46	306	73.88	250.07	114.79	0.00
Serum ferritin	311.84	255.85	200.5	201.49	192.29	181.56	184.65	180.79	164.52	295.28	0.02
TSAT	32.981	15.56	*27.01*	12.68	24.40	8.93	24.22	8.64 2	4. 18	6.91	0.05
Serum albumin-creatinine ratio	48.48	144.71	180.83	626.64	172.86	233.83	524.55	724.71	-	-	0.51
FBS	4.54	0.67	5.03	0.92	5.2	2.29	5.47	1.91	5.26	1.57	0.49
Serum calcium	8.17	1.14	9.06	0.84	9.72	1.047	9.57	1.37	8.85	1.51	0.00
Serum phosphate	3.21	0.63	3.6	0.71	3.76	0.79	3.9	0.93	4.95	1.64	0.00
Serum uric acid	3.88	0.85	4.82	1.47	5.95	1.56	6.89	2.33	6.58	2.02	0.00
TG	134.36	69	174.58	92.91	180.64	124.24	178.72	103.8l	157.15	10l.38	0.23
Cholesterol	161.57	46.3	181.27	48.76	173.35	64.39	175.41	66.15	154.72	75.09	0.15
HDL	37.98	7.52	37.49	5.8	42.98	13. 19	41.15	9.97	13. 19	7.61	0.001
LDL	91 .72	37.53	101.8	30.37	95.14	49.44	94.5	39.35	79.23	55. 16	0.27
PTH	27.34	13.82	28.17	44.45	73 .95	78. l 5	98.74	l33.42	187.82	172.5	0.00
eGFR	113.02	22.83	74.57	7.79	45.59	9.02	22.15	4.18	7.31	3.33	0.00

Serum iron, serum ferritin, TIBC, TSAT and vitamin D, Hb found to be inversely associated and Serum PTH was positively associated with FGF 23 level, with the p value of>0.05.

Table VI: Correlation of FGF23 with various biochemical parameters in CKD patients

Variables	r value	p value
Serum iron	0.021	0.04
TIBC	-0.84	0.00
Serum ferritin	-0.98	0.02
Haemoglobin	0.07	0.43
TSAT	·0.06	0.05
Serum albumin• creatinine ratio	0.81	0.59
FBS	-0.14	0.17
Serum calcium	0.03	0.66
Serum phosphate	-0.46	0.58
Serum uric acid	0.12	0.39
TG	0.63	0.51
Cholesterol	0.10	0.92
PTH	0.12	0.01
Vitamin D	-0.62	0.05
eGFR	0.08	0.46

Pearson correlation test was done to measure the level of significance

Table VII shows the mean difference of serum iron, serum ferritin, Hb, TIBC, TSAT and serum PT between the FGF-23 quartile groups were significant.

Table VII: Biochemical parameters of patients according to FGF-23 quartiles

Variables	1st Quartile		2nd Quartile		3rd Quartile		4th Quartile		p value
	Mean	SD	Mean	SD	Mean	SD	Mean	SD	
Serum iron	98.80	50.66	86.95	28.29	79.19	34.30	76.00	29.73	0.05
TIBC	366.71	82.58	349.75	154.0 1	236.42	l03.07	207.95	87.70	0.01
Serum ferritin	287.72	211.08	209.27	230.34	174.80	247.12	144.22	167.86	0.01
Haemoglobin	12.3	l.8	12.1	1.9	11.9	2.1	11.7	1.8	0.02
TSAT	29.27	12.26	27.13	8.5 1	25.40	9.82	23. 33	13.17	0.00
Serum albumin-creatinine ratio	29.42	55.23	29.50	34.95	55.01	130.16	229. 19	401.28	0.24
Serum calcium	8.77	1.23	9.72	1.40	9.30	1.39	9.54	0.93	0.73
Serum phosphate	3.78	0.97	4.11	0.76	3.91	0.99	4.28	1.75	0.5
Serum uric acid	4.73	1.52	4.99	1.54	4.56	1.50	5.87	0.73	0.52
TG	184.86	97.94	1 41.76	63.46	189.04	111.88	190. 71	79.93	0.42
Cholesterol	157.15	71 .92	173.40	92.37	190.12	69.06	169.17	59.56	0.76
HDL	32.34	9.31	38.28	10.51	37.31	7.66	35.86	4.16	0.46
PTH	160.93	163.86	142.48	112.62	134.76	131.62	110. 79	189.73	0.01
Vitamin. D	16.73	5.04	17.31	7.38	21.37	12.60	18.42	5.16	0.04

ANOVA test was done to measure the level of significance.

Multi variable linear regression analysis of clinical and biochemical parameters associated with level of transferrin saturation showed that serum transferrin saturation was significantly correlated with patient's sex. serum phosphate level, serum iPTH level, vitamin D and FGF23 level.

Table VIII: Multivariable linear regression analysis of clinical and biochemical parameters associated with level of TSAT

Variables	r value	p value
Age	0.134	0.317
Sex	-0.441	0.01 l
Height	-0.261	0.194
Weight	-0.031	0.929
Serum calcium	-0.0 1 0	0.921
Serum phosphate	0.2 17	0.039
Serum uric acid	0.103	0.513
TG	0.053	0.679
PTH	0.297	0.027
Vitamin D	0.063	0.03
Cholesterol	0.059	0.634
HDL	-0.020	0.872
FGF23	0.063	0.04
eGFR	-0.207	0.031

Multivariable linear regression analysis was done to measure the level of significance.

DISCUSSION

Anemia is a common complication of CKD that increases in frequency and severity as eGFR declines [3, 6]. Fibroblast growth factor-23 (FGF23) is a recognized biomarker of adverse outcomes in patients with chronic kidney disease (CKD) [9]. Several cross-sectional studies have suggested a possible association between FGF23 and anemia in CKD patients.

Inverse relationship between serum FGF23 and hemoglobin in patients with non dialysis CKD have been observed in a recent prospective longitudinal cohort study done by Nam *et al.,* on 2,089 patients, showed that FGF23 was independently associated with anemia. In addition, the longitudinal observation of the CKD cohort study revealed that, future development of anemia increased in patients with high FGF23 level. Another cohort study observed that, elevated FGF23 is associated with prevalent anemia, change in hemoglobin over time, and the development of anemia in CKD patients [10]. Though in a study by Akalin *et al.,* they found no association between FGF23 and hemoglobin levels in 89 CKD patients [11]. As well as. a latest observational study by Honda *et al.,* on 282 CKD patients also couldn't establish any significant relationship between FGF23 and hemoglobin levels [12].

Serum iron. serum ferritin. TIBC. TSA T, Serum calcium. Serum phosphate, Serum uric acid,

HDL, Serum PTH were found to be significantly correlated and eGFR inversely correlated with the progression of CKD stages. Multivariable linear regression analysis of clinical and biochemical parameters associated with level of transferrin saturation showed that serum transferrin saturation was significantly correlated with patient's sex. serum phosphate level, vitamin D level, serum iPTH level and FGF23 level.

Studies have showed that. vitamin D deficiency is a risk factor for anemia in patients with CKD. Additionally, vitamin D deficiency is associated with secondary hyperparathyroidism, which can induce bone marrow fibrosis and suppress erythropoiesis in CKD32. This study also found, vitamin D deficiency was inversely associated with the transferrin saturation and increased level of PTH. The understanding frum this study or the relationships between established and non-established risk factors of anemia in *CKD* patients are important to evaluate as, anemia increases the risks of cardiovascular events and mortality and also leads to decreased quality of life.

CONCLUSION

This study found the disrupted iron metabolism and high FGF23 levels. Clinical and laboratory findings have revealed significant inverse relation between FGF23 and serum iron, serum ferritin, serum TIBC and TSAT. The study findings are of great clinical importance, as anemia is a frequent complication in patients with CKD and is associated with adverse outcome.

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