

CORRELATION BETWEEN CHRONIC KIDNEY DISEASES AND HEMATOLOGICAL DATA IN SABRATHA HOSPITAL IN LIBYA

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Received: 09 October 2016, Revised and Accepted: 10 November 2016

ABSTRACT

Objectives: In chronic kidney failure, there is impairment in the excretion of toxic non-volatile solutes, with consequent increase in the plasma concentrations of all metabolites derived mainly from protein metabolism, characterized by increased urea and creatinine, the prevalence and severity of anemia are related to the kidney disease stage and the relative deficiency or reduction in erythropoietin production is the main cause. This study aimed at checking the correlation between anemia and chronic kidney diseases in patients who underwent hemodialysis in Sabratha Hospital in the West of Libya.

Methods: This study was conducted on 60 patients (36 males and 24 females) with chronic renal failure from October 2015 to April 2016 and a group of 40 (20 males and 20 females) individuals as control.

Results and Conclusion: The results of the study concluded that there is a correlation between progression of chronic kidney diseases and reduction in hemoglobin, red blood cells count, hematocrit, and serum iron.

Keywords: Chronic kidney diseases, Chronic kidney disease, Anemia, Renal failure.

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INTRODUCTION

The kidneys function as filters of the blood, removing waste products and controlling the balance of fluid and electrolytes. Filtration occurs via bundles of capillaries called glomeruli. A reduction in the glomerular filtration rate (GFR) to >60 mL/minutes/1.73 m² indicates chronic kidney disease (CKD), as do structural or functional renal abnormalities, which may be present in people with normal GFR [1]. Cross-sectional estimates of the prevalence of CKD in the United States range from 1.5% to 15.6% [2].

Chronic kidney disease is a worldwide public health problem. Major outcomes of CKD include progression of CKD to end-stage renal disease, development of different complication due to impair kidney function, and increased risk for development of cardiovascular disease (CVD) [3]. One of the common complications of CKD is the anemia which is associated with increased risk for CVD, increased morbidity and mortality, especially in high-risk group [4]. The National Kidney Foundation (NKF) defines anemia in CKD as a hemoglobin (Hb) level >13.5 g/dl in men and 12.0 g/dl in women [5]. Anemia is common in diabetic patients with CKD [6]. It is estimated that one in five patients with diabetes and stage 3 CKD have anemia, and its severity worsens with more advanced stages of CKD and in those with proteinuria [7-9].

The prevalence and severity of anemia are related to the kidney disease stage and the relative deficiency or reduction in erythropoietin (EPO) production is the main cause, because the kidneys produce this hormone that stimulates red blood cell (RBC) production and when the patient develops CKD, he/she does not produce it in sufficient amounts. In addition of EPO deficiency, other situations may contribute to the occurrence of anemia in CKD, such as iron, folic acid and vitamin B₁₂ deficiency; blood loss; hemolysis, hyperparathyroidism and inflammation, and these should be investigated before the introduction of EPO replacement therapy [10,11].

In chronic kidney failure, there is also impairment in the excretion of toxic non-volatile solutes, with a consequent increase in the plasma concentrations of all metabolites derived mainly from protein metabolism, characterized by increased urea and creatinine [12]. Creatinine dosage can be used as an aid in kidney function diagnosis, it is most useful and a more sensitive and specific indicator than urea [13]; however, other markers of renal function and renal damage have been investigated and may be introduced in clinical practice to assist in the diagnosis, monitoring, analysis, and prognosis of kidney disease progression [14].

Decline in GFR is associated with a step-wise increase in all-cause mortality at all levels of kidney function [15]. The vast majority of individuals with CKD will experience death due to cardiovascular causes rather than progress to end-stage renal disease or dialysis [16]. The high burden of CVD in CKD cohorts, and vice versa is a reflection of the fact that both disease processes share similar risk factors. Having one disease increases an individual's risk of having the other; the combination of both is associated with a higher mortality than either disease alone [17-19]. This observation is consistent across a number of studies including subgroup analyses from the Antihypertensive and Lipid-Lowering Treatment to Prevent Heart Attack Trial [20]. CV end points, including myocardial infarction, stroke, need for revascularization, the presence of coronary artery disease and all forms of atherosclerotic vascular disease, were increased in study participants as GFR decreased. The presence or absence of anemia appears to add another layer of complexity to the relationship between CKD and CVD. Among individuals with CKD, at all levels of GFR, anemia portends a poor prognosis and is associated with increased mortality compared with those individuals with preserved Hb [21].

Anemia in CKD is associated with cognitive impairment, sleep disturbances, CKD progression, cardiovascular comorbidities, and higher mortality [22,23]. Direct healthcare costs are higher in CKD

patients with anemia than in those without [24], and quality of life issues (e.g., fatigue, reduced productivity) are common [22,23]. Anemia causes fatigue, reduced exercise capacity, reduced libido, and cognitive function, which ultimately have a negative impact on their quality of life [25,26] in addition to being related to heart failure, CVDs are the leading causes of mortality in CKD [27]. Thus, RBC indices, serum iron, transferrin, and ferritin saturation, among others, are tests that may be part of the clinical investigation and monitoring of patients with CKD [11]. Whereas anemia is one of the main consequences of CKD and when found, requires proper treatment and monitoring, checking the tests results can characterize the hematologic changes, iron behavior dynamics, as well as urea and creatinine concentrations and their possible relations with anemia in CKD patients on hemodialysis. Thus, this study aimed at checking the correlation between anemia and chronic kidney diseases in patients who underwent hemodialysis in Sabratha Hospital in the West of Libya.

METHODS

This study was conducted on 60 patients (36 males and 24 females) with chronic renal failure from October 2015 to April 2016 and a group of 40 (20 males and 20 females) individuals as control. Ethical approve and patients consent statement were taken from everyone, and the study was performed in Sabratha Hospital in the West of Libya. At first, all patients with proven chronic renal failure were included in the study. Patients with especial established disorders such as endocrinopathies, and hepatosplenomegaly and also patients with use of certain drugs such as heparin were excluded from the study. During the study, no patient had blood or blood components such as fresh frozen plasma and platelet transfusion. To eliminate effects of sex and age on a comparison between cases and control groups, age and sex were chosen in each pair of groups as similar as possible. Demographic and anthropometric data including age, sex, weight, height, body mass index, and blood pressure were measured for the participants. All of patients and normal participants were Libyans, above 18 years of age, and free from chronic degenerative diseases such as cancer or peritonitis.

A volume of 5 ml of blood was drawn by venipuncture. Collected blood sample was divided into two vials, that is, in ethylenediamine tetraacetic acid vial for hematological tests and plain vial for biochemical test. After clotting of blood in the plain vial, serum was separated, within an hour; by centrifugation at 3000-5000 g for 5 minutes. Serum was used for measurements of urea, creatinine, uric acid, and iron levels. Laboratory standard operation procedures were maintained for all laboratory analysis. Internal quality control sera, both normal and pathological, were also run for each lot of the test, for the validation of the results. Peripheral blood baseline parameters were measured using Sysmex KX 21 analyzer. Biochemical studies were performed using commercially available kits from Biomerieux (France), and serum levels of creatinine, urea, uric acid, and iron were quantified according to the manufacturer's instructions.

Defining variables

Anemia was defined as Hb level <13.0 g/dL for men and <12.0 g/dL for women as per the World Health Organization guidelines. CKD was defined as reduced excretory function with an estimated GFR (eGFR) <60 ml/minutes/1.73 m² as a marker of kidney dysfunction. Furthermore, CKD was defined and classified into five stages of CKD as per NKF guidelines. The formula of Cockcroft and Gault equation was used to calculate eGFR [28].

$eGFR \text{ (in male)} = (140 - \text{age [in years]}) \times \text{weight (in kg)} / (72 \times \text{serum creatinine [mg/dl]})$.

A companion equation for women, based on their 15% lower muscle mass (on average):

$eGFR \text{ (in female)} = (140 - \text{age [in years]}) \times \text{weight (in kg)} \times 0.85 / (72 \times \text{serum creatinine [mg/dl]})$.

Statistical analysis

The data were analyzed using Excel 2010, and GraphPad Prism software version 5. Association between anemia and chronic kidney disease was tested by Pearson's correlation test. Comparison of mean value of continuous data was tested by t-test and ANOVA test. p-value of <0.05 (two-tailed) was used to establish statistical significance.

RESULTS

In this study, sixty patients (36 males, and 24 females) were classified according to their eGFR using of Cockcroft and Gault equation into three categories for each gender, stage III, stage IV, and stage V. In stage III, GFR from 30 to 59 ml/minutes, in stage IV, from 15 to 29 ml/minutes, and in stage V, the GFR is <15 ml/minutes. In our study, most of the patients within stage V limits, as illustrated in Fig. 1, 64% of males and 50% of females. Stage IV patients are 25% of males and 33% of females, and the patients who within stage II limits are only 11% for males, and 17% for females.

The patients also were classified according to their blood groups, we found that most of the patients were have blood Group O, 58% for males and 83.2% for females, and blood Group A were 27.7% for males and 12.5% for females, the percentages of patients with blood Group B were 11.1% for males and 4.1% for females, but there were no any female has blood Group AB, and there were only 2.8% of males who have this blood group (Fig. 2).

The kidney function tests which carried for these patients are, urea, creatinine, and uric acid in serum, and GFR was estimated for each patient using the formula of Cockcroft and Gault equation, by using the collected data of age, weight, and sex for each patient. These measured parameters, as illustrated in Table 1, showed that highly significant increase in serum creatinine in all stages of chronic renal

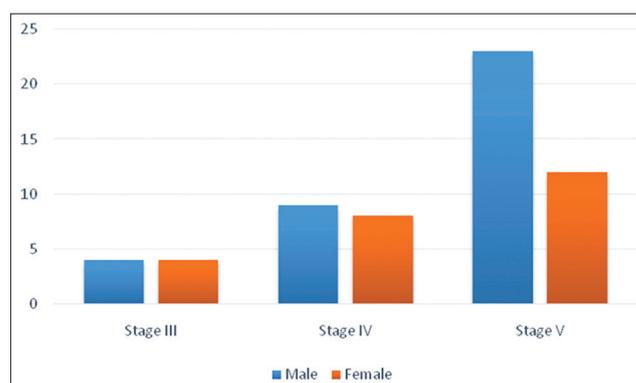


Fig. 1: Distribution of chronic kidney disease patients according to stage of disease

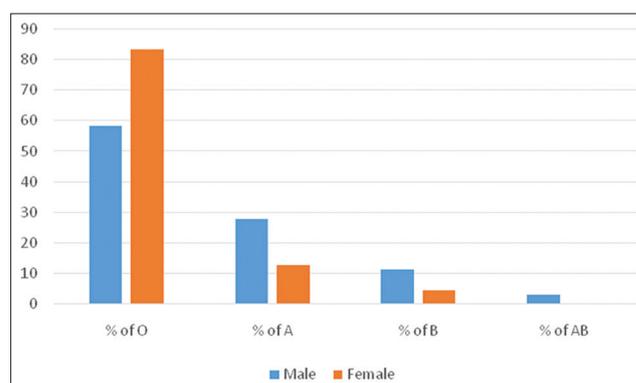


Fig. 2: Distribution of chronic kidney disease patients according to blood groups

Table 1: Kidney function tests in healthy persons and chronic kidney disease patients

Patient groups	Gender	Mean±SD			
		Urea (mg/dl)	Creatinine (mg/dl)	eGFR (ml/minutes)	Uric acid (mg/dl)
Control	Male	25.8±9.05	0.93±0.11	120±15.85	4.8±0.58
	Female	27.3±10.2	0.75±0.09	121±13.33	4.9±0.61
Stage III	Male	293.5**±227.0	3.135**±0.5162	40.04**±6.336	8.225**±1.096
	Female	207.0**±104.7	4.550**±1.485	29.79**±8.167	6.750*±3.182
Stage IV	Male	287.7**±78.58	6.610**±1.259	18.28**±1.875	6.920*±1.963
	Female	291.1**±81.32	6.213**±0.9326	18.12**±2.507	7.213**±2.319
Stage V	Male	261.5**±45.59	9.720**±2.642	11.46**±2.477	6.130*±1.616
	Female	275.8**±68.58	9.320**±2.795	11.59**±2.566	6.302*±2.418

*Significant difference compared to control group ($p < 0.05$). **Highly significant difference compared to control group ($p < 0.01$). SD: Standard deviation, eGFR: Estimated glomerular filtration rate

failure in male patients by 273%, 610%, and 945% in stages III, IV, and V patients, respectively. The increase of serum creatinine levels in female patients were, 506%, 728%, and 1142% in stages III, IV, and V patients, respectively, as compared to control group. Serum urea levels were also increased significantly in all stages of chronic renal failure in male patients by 1037%, 1015%, and 913% in stage III, IV, and V patients, respectively, and the female patients showed highly significant elevations in serum urea by 658%, 966%, and 910% in stage III, IV, and V patients, respectively, as compared to healthy persons. The eGFR in all patient groups was decreased with highly significant degrees, as illustrated in Table 1. It decreased in male patients by -67%, -84%, and -90% in stage III, IV, and V patients, respectively. This parameter was reduced in female patients by -75%, -85%, and -90% in stage III, IV, and V patients, respectively, as compared to control persons. Serum uric acid was increased also by significant in male patient groups by 71%, 44%, and 27% in stage III, IV, and V patients, respectively, whereas, its changes in female patients were 40%, 50%, and 31% in stage III, IV, and V patients as compared to healthy control group (Table 1).

Measurements for peripheral blood were carried out for RBCs, white blood cells (WBCs), and platelets count, Hb concentration, hematocrit, and serum iron were also measured, mean corpuscular hemoglobin (MCH), MCH concentration (MCHC) and mean corpuscular volume (MCV) were calculated. The hematological data were tabulated in Table 2 which showed significant changes compared to healthy control group, these changes were appeared as decrease in Hb concentrations in male patients by -17%, -18%, and -30% in stage III, IV, and V patients, whereas the changes in this parameter in female patients are -11%, -20%, and -26% in stage III, IV, and V patients as compared to control. RBCs count was also decreased in all patient groups with statistically significant degrees, as illustrated in Table 2. The changes in RBCs count in male patients are -34%, -38% and -38% in stage III, IV, and V patients, respectively, whereas, these changes in females are -21%, -28%, and -28% for stage III, IV, and V, respectively as compared to healthy women. Hematocrit percentage were also reduced in all patient groups by -37%, -46%, and -38% in male patient groups of stages III, IV, and V, respectively, whereas, in female patients the percentages of reduction in this parameter are -30%, -36%, and -32% in stage III, IV, and V patients.

Serum iron levels were also reduced by -34% and -42% in stage IV and V patients respectively, but there is a significant change in stage II male patients. In female, the degrees of reduction in serum iron are -29% and -41% in stage IV and V patients. Platelets count was also decreased in patient groups by -38% and -26% in stage V male and female patients, respectively.

The data in Table 3 show the correlation between the changes in kidney function tests and hematological parameters. This correlation was appeared between serum creatinine and Hb, RBCs count, hematocrit, MCH, MCHC, and serum iron, on the other hand, no correlation between creatinine and WBCs or platelets count. In this table also, the correlation between serum urea concentration and blood data were illustrated. These

data showed a correlation between serum urea and Hb and RBCs count but no correlation between urea and other hematological data. eGFR also showed correlation with Hb, RBCs count, MCHC, and serum iron.

DISCUSSION

Chronic kidney disease is a major public health problem and a major cause of morbidity and mortality worldwide. CKD is diagnosed on the basis of the presence of markers of kidney damage and kidney function. This study was carried out to assess the hematological profile in CKD including the Hb, RBCs count, WBCs count, platelets count, hematocrit, MCH, MCHC, MCV, and serum iron and their correlation with renal function tests.

Anemia in CKD is typically normocytic, normochromic, and hypoproliferative. The demonstration of a circulating factor responsible for stimulating erythropoiesis and the kidney as the main source of EPO in the 1950s [29,30] engendered the hypothesis that EPO deficiency is a predominant cause of anemia in CKD.

Numerous studies suggest that circulating uremic-induced inhibitors of erythropoiesis contribute to the anemia, shortened RBC survival also contributes, as demonstrated by radioisotope labeling studies. Although the etiology is not entirely clear, metabolic, and mechanical factors have been proposed [31,32]. In this study, reduction in Hb, RBCs count, and hematocrit is in accordance with the study of Poudel *et al.* [33], Bueno and Frizzo, [34] and de Francisco *et al.* [35].

Iron deficiency in the general population is a common cause of anemia and is prevalent in patients with diabetes and CKD. In these same patients, dietary deficiency, low intestinal absorption, and gastrointestinal bleeding may result in absolute iron-deficiency anemia. Recent analyses of the National Health and Nutrition Examination Survey IV suggest that up to 50% of patients with CKD stages 2-5 have absolute or relative (functional) iron deficiency [36]. In CKD, both absolute and relative iron deficiency are common. Absolute iron deficiency is defined as a depletion of tissue iron stores evidenced by a serum ferritin level >100 ng/ml or a transferrin saturation of $>20\%$. Functional iron deficiency anemia is adequate tissue iron defined as a serum ferritin level >100 ng/ml and a reduction in iron saturation. The latter is more common and is strongly associated with upregulation of inflammatory cytokines and impaired tissue responsiveness to EPO, which can inhibit iron transport from tissue stores to erythroblasts [37]. Increased levels of inflammatory cytokines such as interleukin-6 enhance production and secretion of hepcidin. Hepcidin is the main hormone responsible for maintaining systemic iron homeostasis, produced by the liver and secreted into circulation, hepcidin binds and induces degradation of the iron exporter, ferroportin, on duodenal enterocytes, reticuloendothelial macrophages, and hepatocytes to inhibit iron entry into the plasma. Inflammatory cytokines directly induce hepcidin transcription, presumably as a mechanism to sequester iron from invading pathogens, leading to the iron sequestration, hypoferrinemia, and anemia that are hallmarks of many chronic diseases including CKD. Numerous studies show that hepcidin is elevated in CKD patients. Mechanisms suggested

Table 2: Hematological parameters in healthy persons and chronic kidney disease patients

Patient groups	Gender	mean±SD	RBCs (mill/ μ l)	Hb (g/dl)	MCV (fl)	Hematocrit (%)	MCH (pg)	MCHC (g/dl)	WBCs ($\times 10^3$ / μ l)	Platelets ($\times 10^3$ / μ l)	Iron (μ g/dl)
Control	Male	4.5±0.523	13±0.82	92±5.55	45.5±5.23	30.56±3.33	35.25±2.45	7.27±1.55	252±50.52	120±25.55	
	Female	3.9±0.48	12.5±0.62	93±6.26	39.3±6.33	29.5±5.21	34.5±3.23	7.42±1.23	250±55.62	109±22.12	
Stage III	Male	2.970**±0.2546	10.75**±0.9192	95.40±1.125	28.65**±2.333	36.46±6.223	37.75±6.293	6.200±1.697	214.5±12.02	116±39.84	
	Female	3.06**±0.3818	11.1*±0.4243	96.95±3.323	27.50**±0.7071	36.65±5.961	40.40±2.546	6.150±1.768	213.5±13.44	102±15.59	
Stage IV	Male	2.789**±0.4629	10.66**±1.147	91.53±5.661	24.50**±4.753	38.83±5.151	46.08±15.46	6.890±2.148	155.4**±71.00	78.64*±21.61	
	Female	2.793**±0.3078	9.913**±1.648	95.06±8.123	25.00**±2.426	35.83±7.228	40.09±8.411	6.675±1.871	193.6±68.46	77.89**±24.09	
Stage V	Male	2.802**±0.7221	9.152**±1.986	88.35±11.30	27.85**±5.915	33.70±7.271	33.41±6.608	6.817±2.221	210.2±62.35	68.57**±24.66	
	Female	2.812**±0.5696	9.3**±1.950	95.30±5.393	26.73**±3.810	33.56±6.675	34.82±5.792	7.008±2.122	182.8**±49.08	64.36**±27.57	

*Significant difference compared to control group (p<0.05), **Highly significant difference compared to control group (p<0.01). RBC: Red blood cell, MCV: Mean corpuscular volume, MCH: Mean corpuscular hemoglobin, MCHC: Mean corpuscular hemoglobin concentration, WBC: White blood cell, SD: Standard deviation

Table 3: Correlation between kidney function tests and hematological data in chronic kidney disease patients

Parameter	Hb	RBCs	Hematocrit	WBCs	MCV	MCHC	MCH	Platelets	Iron
Correlation of creatinine with hematological data									
Pearson r	-0.424	-0.367	-0.4217	-0.105	-0.121	-0.272	-0.337	-0.0705	-0.2991
p value (two-tailed)	0.0009	0.0046	0.0010	0.4326	0.3825	0.0386	0.0096	0.5989	0.0349
p value summary	***	**	***	ns	ns	*	**	ns	*
Correlation of urea with hematological data									
Pearson r	-0.348	-0.293	0.019	-0.0613	-0.01	-0.14	-0.124	-0.0085	0.1877
p value (two-tailed)	0.0074	0.0256	0.8874	0.6473	0.9427	0.2947	0.3523	0.9492	0.1918
p value summary	**	*	NS	NS	NS	NS	NS	NS	NS
Correlation of eGFR with hematological data									
Pearson r	0.3832	0.2954	0.01421	0.05451	0.09044	0.2831	0.2513	0.05444	0.2807
p value (two-tailed)	0.0030	0.0244	0.9157	0.6845	0.5154	0.0313	0.0571	0.6848	0.0398
p value summary	**	*	NS	NS	NS	*	NS	NS	*

*Significant difference compared to control group (p<0.05), **Highly significant difference compared to control group (p<0.01***??). RBC: Red blood cell, MCV: Mean corpuscular volume, MCH: Mean corpuscular hemoglobin, MCHC: Mean corpuscular hemoglobin concentration, WBC: White blood cell, eGFR: Estimated glomerular filtration rate, NS: Not significant

to account for this are increased expression by inflammatory cytokines and reduced renal clearance [38].

Based on its ability to donate and accept electrons, iron is essential for many important biologic reactions, including oxygen transport, cellular respiration, and DNA synthesis. However, this same property makes excess iron toxic by generating free radicals that can damage or destroy cells. Systemic and cellular iron levels must, therefore, be tightly regulated. The majority of iron (20-25 mg) is provided by recycling from senescent RBCs, which are phagocytosed by reticuloendothelial macrophages to store iron until it is needed, with lesser amounts provided by dietary absorption in the duodenum (1-2 mg) and release from liver stores. Plasma iron, which circulates bound to transferrin, is relatively limited at 3 mg, and therefore must be turned over several times to meet the daily requirements for erythropoiesis. With no regulated mechanism for iron removal, typical iron losses are 1-2 mg daily, mainly from intestinal and skin cell shedding and menstruation in reproductive-age women. Systemic iron balance is, therefore, maintained by regulating dietary iron absorption and iron release from storage sites in the liver and reticuloendothelial macrophages [39]. In the current study, serum iron level was reduced significantly in stage V patients of CKD, and this in accordance with the previous studies of Mezzano *et al.* [37] and Babitt and Lin [39].

Both deficiency and hyporesponsiveness to EPO contribute to anemia in diabetic patients with CKD. The cause of EPO deficiency in these patients is thought to be reduced renal mass with consequent depletion of the hormone. Hyporesponsiveness is defined clinically as a requirement for high doses of EPO to raise blood Hb level in the absence of iron deficiency. It is believed to represent impaired antiapoptotic action of EPO on proerythroblasts. Possible causes of this EPO hyporesponsiveness include systemic inflammation and microvascular damage in the bone marrow [9]. However, some studies suggest that other factors (i.e., autonomic failure) may play a role in impaired EPO production or secretion by failing kidneys.

Hassoon *et al.*, [40] found that the percentage of the renal failure patients that carried O blood group was the highest (55%) followed by the B blood (25%), A blood group (12.5%) and AB blood group (9.4%) [40]. In another study, the occurrences of renal failure in Group O were 69.5%, and Group B was 42%, whereas AB was zero and 69% was for A blood group [41]. In our study, about 65% of CDK patients with blood Group O, which in accordance with the previous studies. Dorgalaleh *et al.*, [42] reported that platelet count was statistically significant decreased and mild thrombocytopenia in chronic renal failure patients. Although the mean platelet count did not show that our patients have not in a potential bleeding risk thrombocytopenia was an important risk factor for the occurrence of bleeding among a minority of our study patients. Furthermore, authors were found a mild thrombocytopenia among chronic renal failure patients.

CONCLUSION

This study concluded that there is a correlation between progression of chronic kidney diseases and reduction in Hb, RBCs count, hematocrit, and serum iron.

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Author Query???

AQ1: Kindly provide significant value