Original Article

Erythropoietin levels in geriatric anemia

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Abstract

Background: Defects in the production or action of erythropoietin (EPO) are important contributing factors in anemia. However, the exact impact of aging on levels of EPO and its role in the development of geriatric anemia is still underexplored. Moreover, the specific pattern of EPO in etiological subcategories such as nutritional anemia (NA), anemia of chronic disease (ACD), and unexplained anemia (UA) is not entirely known. **Objective:** The aim of the study was to determine the serum EPO levels in geriatric anemia and compare them across NA, ACD, UA, and NA with ACD. **Materials and Methods:** Ninety anemic geriatric patients (cases) along with 30 non-anemic geriatric controls were evaluated for serum EPO levels. A correlation between S.EPO and inflammatory markers was also done. **Results:** Serum EPO levels were higher in cases as compared to controls ($P < 0.00$). After adjusting for outliers, the reference range of EPO in controls was the same as in normal young adults (2.21–20.95 mU/mL). The majority (37/58, 63.7%) of NA patients had increased S.EPO levels (highest among all four subcategories and controls). S.EPO also correlated inversely with high-sensitivity CRP (hsCRP) and serum ferritin (SF), reinforcing that the inflammatory state suppresses S.EPO levels. **Conclusion:** Geriatric anemic patients have elevated S.EPO as compared to non-anemic controls (observed reference range similar to young adults). Raised EPO levels were detected more frequently in NA, while they were the lowest in UA.

Keywords: Erythropoietin, geriatric anemia, inflammatory markers, unexplained anemia

Introduction

Geriatric anemia (GA) is a multifactorial condition with a rapidly increasing prevalence.[1–7] It is a major cause of poor quality of life and increased mortality in these patients. Most of the time, GA is treatable and can significantly improve the quality of life. Its main etiological subcategories include nutritional anemia (NA), anemia of chronic disease (ACD)/anemia of inflammation (AI), and unexplained anemia (UA).[2,4,5,8–15] There are different postulated pathophysiological mechanisms giving rise to GA. One such important mechanism is low production of erythropoietin (EPO) and/or hyporesponsiveness to EPO. It is a key hormone regulating erythropoiesis and is primarily produced by kidneys.[10,12,16–18] Its levels are known to vary with age, gender, and physiological conditions. It is also known to be increased in response to anemia. However, the degree of response varies with the etiology of anemia. Hence, the reference range of EPO in adults is variably reported. According to national committee for clinical laboratory standards (NCCLS), the normal range is 3.22–31.9 mIU/ml.[19] Another study observed median values of 7.6 (5.8–9.9) mIU/mL in males and 7.9 (6.0–10.6) mIU/mL in females.[20] Charuruks N *et al.*[21] demonstrated a range of 1.76–25.29 mU/mL for males and 2.82–16.68 mU/mL for females. Additionally, higher EPO levels are reported in healthy geriatrics as compared to those in young adults.[10,20,21]

However, the physiological effects of aging on EPO and its regulating factors are not well known. Some of the very first

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studies found that EPO synthesis increases with age.[22–25] High EPO levels could result from age-related impairment in hypoxia/EPO sensing, and increased EPO resistance of RBC precursors.[22,23,26] or physiological response to chemically increased hypoxic stimuli due to an undiagnosed disease.[5,23,26] It may rise in the geriatric population without chronic renal failure (CRF) as well.[23–25,27] On the contrary, others have detected lower EPO levels in non-anemic geriatric patients compared to healthy younger persons.[12,28,29] Elevation in pro-inflammatory cytokines during aging is also associated with reduced EPO.[5,22,23,30] Yet, a few authors failed to find any correlation between EPO levels and aging.[20,22,31] Deranged EPO levels have significant clinical implications. While higher EPO levels in geriatrics have been linked to higher creatinine clearance, elevated C-reactive protein levels, cardiovascular diseases, and increased mortality, low EPO can cause anemia without advanced renal failure as well.[32-35]

With respect to etiological subcategories of GA, EPO levels seem to be affected variably in NA, ACD, and UA. While this has been extensively studied in children and young adults, there is limited data for geriatrics. Irrespective of underlying mechanisms, higher EPO levels have been found in non-geriatrics with NA.[27] Furthermore, EPO levels shoot up in iron deficiency anemia (IDA).[12,36] However, some studies also reported lower EPO levels in geriatric NA.[23,36,37] Universally, low EPO is detectable in benign etiology of GA, that is, ACD/AI and UA.[4,36] Gowanlock *et al.*[36] suggested lower EPO levels in UA than IDA and ACD despite adjusting for hemoglobin (Hb), estimated glomerular filtration rate (eGFR), and comorbidities, indicating primary EPO underproduction over bone marrow suppression as the reason for anemia. Several comparative studies on EPO levels in non-anemics, AI, or NA showed ambiguous results.[3,5,10,14,36,38,39] A clear understanding of the underlying processes in this heterogeneous group is pivotal in the management of GA. This could help the clinician understand the response of geriatric patients to standard treatment regimens and tailor further management of these patients.

In view of the ambiguous data on EPO levels in GA and the paucity of literature, particularly from India, the current study was undertaken to primarily determine the serum EPO levels in patients with GA and further compare their levels in NA, ACD, UA, and NA coexisting with ACD and to understand the difference in EPO response to anemia caused by different etiologies, if any.

Materials and Methods

Study design and setting

It was a cross-sectional descriptive study conducted from November 2018 to March 2020.

Ethical approval

Ethical approval from the Institutional Ethics Committee for Human Research (IEC-HR) was obtained.

Study population

All anemic patients, 60 years of age and above, visiting medicine or geriatric OPD were enrolled in the study. Anemia was defined as Hb <12 g/dL in women and <13 g/dL in men.[3,4] Ninety patients who consented to further evaluation were subjected to extensive *hematologic tests* including a complete hemogram (Beckman Coulter LH500) with erythrocyte sedimentation rate (ESR) (Westergren method) and peripheral smear preparation (Wright stain), and *biochemical tests* including iron status [serum iron (SI) [ICSH 1978], total iron-binding capacity (TIBC) [Ressler and Zak], percentage transferrin saturation (TSAT)], serum ferritin (SF) [enzyme-linked immunosorbent assay (ELISA) kit, Calbiotech, Inc.], serum vitamin B12, and folate assay [ELISA kit., Calbiotech, Inc.].[40,41] Serum levels of high-sensitivity CRP (hscrp) [ELISA kit (Diagnostics Biochem Canada Inc.)] were checked considering it as an inflammatory marker and serum EPO [ELISA kit (DRG International, USA)] was measured in all the patients. To prevent any possible bias in EPO values due to physiological circadian variation, early morning fasting venous samples were collected for all subjects. For comparative analysis, 30 non-anemic geriatrics were also recruited. The values of SF >15 μg/L, hsCRP < 6 mg/L, and EPO = 3.22–31.9 mIU/mL were considered normal.

Patients with CRF/known hematological disorders or any malignancies, hematinic intake in the last 10 days, or blood transfusion in the last month were excluded.

After a thorough work-up, four main etiological subcategories, namely, NA (iron, vitamin B12, and folate deficiency), ACD, UA, and NA with ACD were considered according to the following criteria: IDA – low SI (<60 μg/dL) and SF (<15 μg/L), vitamin B_{12} deficiency – <180 ng/L, FA deficiency – <3 μg/L, ACD – low SI (<60 μg/dl), low to normal TIBC (N – 250–400 μg/dL) and SF ≥ 30 μg/dL, UA – when the patient could not be assigned any of the above categories using the above criteria.[2,3,5,14,38]

Statistical analysis

Statistical analysis was performed using MS Excel and SPSS software. Median (IQR)/mean ± SD and range were calculated for quantitative data. Means/medians of inflammatory markers were calculated. A Chi-square test was applied for categorical data. An independent T-test was applied for parametric data. Mann-Whitney test and Kruskal-Wallis test were applied for non-parametric data. *P*-value less than 0.05 was considered significant.

Results

The age of the patients ranged from 60 to 80 years with mean ± SD of 66.1 ± 5.7 years in cases (n = 90) with 48 males and 42 females, and 60–85 years with mean ± SD of 65.3 ± 5.8 years in controls (n = 30) (nine males and 21 females. The majority (68/90, 75.5%) of the patients in these cases were suffering from different illnesses including chronic co-morbid

conditions at presentation and anemia was incidentally detected in them. The remaining patients (22/90, 24.4%) were referred for management of anemia primarily. A complete hematologic evaluation of all patients was done, and different parameters were compared between cases and controls [Table 1]. The most common cause of GA in this study was NA (58/90, 64.4%) followed by ACD (12/90, 13.3%) and NA with ACD (12/90, 13.3%). UA (8/90, 8.8%) was the least common cause. In NA, IDA was the most common cause (22/90, 24.4%). In 36.7% of cases, there was more than one cause of anemia.

Overall, mean serum EPO levels were 165.4 ± 308.7 mIU/mL in cases and 25.9 ± 38 mIU/mL in non-anemic controls (*P*-value < 0.000). The EPO levels (mIU/mL) were further studied across etiological subgroups: NA (mean = 217.4 ± 357.4, median = 74, range = 7–2034), ACD (mean = 57.2 ± 73.7, median = 13, range = 9–192), UA (mean = 36.2 ± 51.0, median = 11.3, range = 1–144), and NA with ACD (mean = 50 ± 61.6, median = 13.8, range = 5–167) [Table 2]. Further, Mann-Whitney and Kruskal-Wallis tests were performed to compare EPO levels between NA, ACD, UA, NA with ACD as well as overall study group for any statistical difference [Table 3].

The inflammatory markers evaluated were ESR, SF, and hsCRP. ESR was raised in 86/90 (95.6%) patients and 25/30 (83.3%) controls. Fifteen (16.6%) patients and 4/30 (13.3%) controls had raised SF. hsCRP was raised in 38/90 (34.2%) patients and 15/30 (50%) controls. Since inflammation is known to suppress serum EPO, their correlation was also studied in some cases. hsCRP and SF were inversely related to serum EPO levels (r values: −0.199 and −0.038, respectively). However, only a trend was seen without any statistically significant correlation. SF and hsCRP were positively correlated with each other, but only a trend was observed (r value: 0.186, *P*-value: 0.079) [Table 4]. Further, Chi-square test was applied to compare the difference in inflammatory markers and normal and high serum EPO in the overall study group [Table 5].

With reference to UA, SF was increased in 6/8 (75%) patients and hsCRP was elevated in 3/8 (37.5%) patients. Serum EPO levels were low in 2/8 (25%) patients, normal in 3/8 (37.5%) patients and elevated in 3/8 (37.5%) patients [Figure 1].

Discussion

Geriatric anemia is a common multifactorial condition in the elderly age group. Various pathophysiological mechanisms act simultaneously, ultimately resulting in GA. These include age-associated progressive decline in bone marrow cellularity leading to diminished regeneration and differentiation of hematopoietic stem cells (HSCs), coupled with low production of EPO and/or hyporesponsiveness to EPO and co-existing pro-inflammatory state (*de novo* or associated with chronic inflammatory disorders) affecting HSC function.[3,5,16–18,30,26] Other contributing factors are low telomerase activity, progressive loss of telomeric DNA, age-related endocrine disturbances (hypothyroidism, low testosterone, and estrogen deficiency), and polypharmacy causing anemia.[3,4,5,7,36,42,43]

EPO is considered to be an important regulator of erythropoiesis. However, there is limited data available regarding its normal range in anemic as well as non-anemic geriatrics. The index study was conducted to study the serum levels of EPO in geriatric non-anemics and anemics, as well as across different etiological subtypes of GA.

In the index study, the majority of non-anemic controls had normal EPO levels similar to the reference range for young adults, and none of them showed low EPO. A similar observation was also made by Kario *et al.* and Beverborg *et al.*[20,21] However,

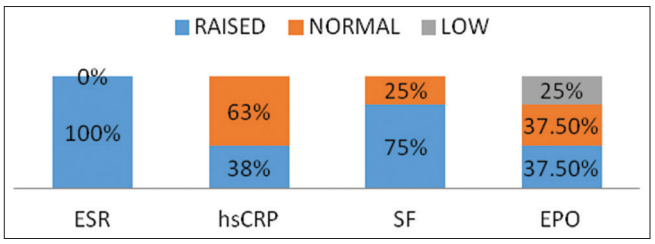

Figure 1: Inflammatory markers and EPO in UA in geriatrics (n = 8). Cut-off values: SF < 15 µg/L, hsCRP < 6 mg/L, S.EPO = 3.22–31.9 mIU/mL. The pattern of rise in levels of inflammatory markers in patients with UA was ESR (100%) > SF (75%) > hsCRP (38%). One-fourth of patients with UA lacked EPO elevation, while the remaining had normal to high values. However, these levels were still lower than non-anemic controls indicating an overall inadequate EPO response in these patients

Table 1: Hematologic parameters in the cases (*n*=90) and controls (*n*=30)

Parameters	Cases		Controls		Significance, *P*
	Mean±SD	Range	Mean±SD	Range	
Hemoglobin (g/dL)	8.4±2.1	4.4–11.8	8.4±2.1	12–18	<0.00*
Hematocrit (%)	26.1±8.8	0.19–47.4	40.5±9	0.34–56.2	<0.00*
Red cell count ($\times 10^{12}$/L)	3.6±0.8	1.1–5.2	4.7±0.9	1.57–5.7	<0.00*
Mean corpuscular volume (fL)	78.4±14.6	54–127	89.5±8.9	73–116	<0.00*
Mean corpuscular hemoglobin (pg)	23.9±5.8	15.3–41.8	28.7±2.9	22.3–35.8	<0.00*
Mean corpuscular hemoglobin concentration (g/dL)	29.7±4.0	24.8–35.3	32.0±1.3	30.1–35.2	<0.00*
Total leukocyte count ($\times 10^9$/L)	8.5±3.16	3.1–20.4	7.9±1.9	3.3–13.7	<0.31
Platelet count ($\times 10^9$/L)	267.7±14.6	1.2–645	206.9±88.2	040–407	<0.67
Reticulocyte count (%)	1.6±0.9	0.2–4	1.60±1.16	0.5–7	<0.82

*Significant differences between the two groups

Guralnik and Balducci *et al.*[31] reported decline in EPO levels with advancing age in non-anemics.[5] A small subset in the index study showed high EPO. This finding is in agreement with other studies that also displayed raised EPO levels in elderly non-anemics.[20,23,24,31] Further evaluation of these subsets of control patients with normal Hb showed low vitamin B12 and laboratory signs of latent iron deficiency and IDA coexisting with ACD. This finding suggests that subclinical levels of nutritional deficiency can also be associated with increased EPO levels. After adjusting for these outliers, the EPO levels in the control group were revised to 7.5–24.5 mIU/mL (mean = 22.3 mIU/mL), which is similar to the normal reference range.

Serum EPO levels were raised in cases as compared to non-anemic geriatric controls, as also observed in other studies by Beverborg *et al.*, Sriram *et al.*, and Gowanlock *et al.*[20,36,37] Serum EPO could rise due to subclinical blood loss, increased RBC turnover or EPO resistance of RBC precursors in geriatrics. Increased EPO levels might also be the initial sign of subclinical disease or developing GA. There is an impaired EPO response to renal diseases and malignancy, therefore, such patients were excluded from the study. Hence, this study represents a true EPO response to anemia, not confounded by renal disease. However, few authors have found reduced EPO levels in GA patients.[22,31,44] Hence, more studies are recommended to establish this observation.

In GA, the majority of NA patients showed higher EPO levels, ranging from 7–2034 mIU/mL with mean ± SD of 217.5 ± 38 mIU/mL (median = 74); this is the highest among all etiological subcategories as well as controls. This further concurs with other studies.[1,12,36] Within NA, EPO levels were uniformly high in IDA patients. A plausible explanation for this finding could be the requirement of iron for the degradation of hypoxia-inducible factor 1 (HIF-1), which consequently induces EPO production and secretion. Thus, IDA induces EPO production.[12,21,34,37,45–47] Yet, an opposite observation was made by Kario K *et al.*,[23] wherein reduced EPO levels were found in IDA patients.[47] Most vitamin B12 deficiency patients showed normal EPO levels. This is contradictory to studies by Kario and Ferruci *et al.*,[23] who observed reduced EPO levels, whereas raised EPO levels were found in vitamin B12 deficiency by other authors.[28,36] Most patients with FA and combined deficiencies of iron and vitamin B12 had elevated EPO. However, the number of patients in each of these nutritional subgroups was less and more studies are warranted to establish a correlation.

Table 2: S.EPO levels in subcategories of GA (*n*=90)

S.EPO (mIU/mL)	NA%	ACD%	UA%	NA + ACD%
Low (<3.22)	0	0	25	0
Normal (3.22–31.9)	36.2	66.6	37.5	66.6
High (>31.9)	63.7	33.3	37.5	33.3

Most ACD patients had EPO levels within the normal range, while the remaining patients showed elevation, with lower mean values than NA but still higher values than controls. But Ferrucci L *et al.*[45] found both rising and declining levels of EPO in ACD patients. While the decline is expected, the elevation in EPO levels could result from co-existing subclinical NA in these patients.

UA was the only etiological subcategory to demonstrate low EPO: the levels were lower than ACD, NA, and NA coexisting with ACD but still higher than controls. While the EPO levels showed a wide range, the median was still in the normal range indicating a suboptimal response of EPO to anemia. This matches the findings by Guralnik and Ferrucci *et al.*[5,45] Researchers have proposed that UA in geriatrics could result from a chronic pro-inflammatory state, impaired renal function, diminished EPO production, or an inadequate bone marrow response to EPO. Low EPO in UA may result from the inhibitory effect of inflammation as well as the co-morbid impact of diabetes and hypertension, both fairly common diseases in geriatrics.[20,21] Other potential pathologic mechanisms behind low EPO in these diseases include loss of hypoxic response as a stimulus for EPO secretion owing to possible functional and/or structural changes in proximal tubules and the cortical interstitium of the kidney, effects of advanced glycation end products, or autonomic dysfunction.[48] Thus, ascending EPO levels in UA may still remain inappropriately low for the requirement, particularly when compared with other etiological forms of GA, as occasionally confirmed in the literature.[5,10,12,14,36,38] Despite these findings, there are studies with contrasting results in UA patients as well.[37]

In NA coexisting with ACD, mean EPO levels were raised. There is no specific literature regarding EPO levels in this category.

Extended analysis of EPO levels between all etiological sub-groups showed that EPO levels (*P*-value < 0.05) were statistically significant when NA was compared with ACD and NA coexisting with ACD. It was significant in the overall group (*P*-< 0.004). A statistically significant difference with UA could not be ascertained, likely due to the lesser number of patients in this category.

Since inflammation suppresses serum EPO, its correlation was also studied with inflammatory markers, namely ESR, SF, and hsCRP. Of these, hsCRP and SF were inversely related to serum EPO. A significant correlation (*P* value < 0.023) was found between SF and S.EPO, pointing toward a suppressive effect of

Table 3: Comparison of S.EPO in NA, ACD, UA, and NA with ACD (*n*=90)

	NA-ACD	NA-UA	NA-NA with ACD	ACD-UA	ACD-NA with ACD	UA-NA with ACD	Overall group
P	<0.05*	<0.15	<0.05*	<0.84	>0.9	<0.49	<0.004*

*Significant difference between the two groups

Table 4: Correlation matrix between inflammatory markers and S.EPO levels in GA (*n*=90)

	SF (µg/L)		HsCRP (mg/L)		EPO levels (mIU/mL)	
	r	*P*	*r*	*P*	*r*	*P*
SF (µg/L)	1		0.186	0.079	−0.199	0.060
hsCRP (mg/L)	0.186	0.079	1		−0.038	0.723
EPO (mIU/mL)	−0.199	0.060	−0.038	0.723	1	

Table 5: Comparison of inflammatory markers and EPO (*n*=90)

Inflammatory markers	EPO (*P*)
ESR (mm/h)	0.770
hsCRP (mg/L)	0.068
SF (µg/L)	0.023*

*Significant difference between the two groups

SF on EPO. Further, SF and hsCRP were positively correlated with each other. The positive association between these two markers hints at their synergistic influence on the inflammatory profiles of GA patients. While the index study failed to derive a statistically significant association, more reliable results were observed by Shastri *et al.*[3] Nevertheless, accurate analysis of inflammation on the serum EPO levels requires large-scale studies incorporating more patients with hyper-inflammation and an expanded panel of pro-inflammatory markers.

The study of EPO levels in GA is vital. Apart from uncovering lacunae in production or response to EPO, assessment of serum EPO can facilitate early distinction of benign ACD/AI and UA from anemia secondary to bone marrow diseases, including malignancies where the EPO production is impaired.[3,10,20,27] Since EPO is known to affect iron metabolism, its derangement can further influence the hematinic response in IDA in geriatrics. Furthermore, knowledge about EPO status can enable treating physicians to decide about the utility of EPO stimulating agents in GA, especially in patients with mild IDA and/or iron-restricted erythropoiesis. Any degree of anemia in geriatrics may signify a more severe underlying disease. Hence, prompt identification and detection of underlying causative factors are crucial for successful management and improving the overall quality of life in geriatrics. Failure to correctly diagnose and treat anemia might result in poor therapeutic outcomes which can even deteriorate other coexisting disease conditions in these patients, if any.[11]

Strengths of study

The index study highlights key findings regarding serum EPO in geriatric anemia in the Indian population. For an unbiased assessment, strict patient inclusion criteria were used, and those with confounding conditions (malignancy and CRF) were excluded from the study cohort, making the results more representative of anemia. The uniformity in sampling time and the nature of venous samples prevented bias due to circadian variation in EPO levels. For a more accurate interpretation, EPO levels were compared with non-anemic controls.

Limitations of the study

This is a hospital-based study and may not be representative of the community as a whole. Also, despite excluding patients with renal disease, renal function tests could not be included to account for subclinical disease. Further, the confounding effect of age-related co-morbidities on inflammatory patterns in subcategories of GA could not be completely excluded by the study design.

Future directions

The index study can provide baseline data and help in initiating further studies on the evaluation of EPO levels in GA. Large-scale explorative studies in hospital settings as well as comparative studies vis-à-vis non-anemic community-based controls may be undertaken for better reflection on EPO status. Further studies may be appropriately designed to account for confounding co-morbidities and other subclinical diseases in a larger number of patients, which could secondarily elevate serum EPO levels.

Summary

The study describes serum EPO levels in non-anemic and anemic geriatric patients in the Indian population. Anemic patients showed elevated levels of serum EPO, while the levels were predominantly normal in non-anemic controls. The values of EPO in non-anemic geriatrics are almost similar to the reference range in young adults (2.21–20.95 mU/mL). Overall, nutritional deficiencies emerged as the top-most cause of anemia in this age group. Raised EPO levels were detected more frequently in NA, indicating a favorable EPO response, while they were suppressed in ACD as expected. Low EPO levels in UA represent a suppressed response, likely due to EPO resistance as a possible mechanism.

Take home message

The normal range of EPO levels in non-anemic geriatric controls (similar to normal young adults) suggests that under normal circumstances, EPO is not physiologically deranged in geriatrics. However, there is always the possibility of non-overt, subclinical diseases in geriatrics, necessitating further studies for validation of these results. EPO levels were raised in anemic geriatrics with NA, unlike UA. These are interesting observations and need to be validated with appropriately designed, larger community-based studies.

Novel findings

To the best of our knowledge, the index study is probably the first such study in Indian geriatrics, specifically assessing serum EPO levels in GA. The status of EPO, specifically across etiological categories of GA with special reference to UA, has not been addressed. No other study so far has taken into account the inflammatory parameters to understand their impact on EPO levels in GA.

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Conflicts of interest

There are no conflicts of interest.

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