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REVIEW ARTICLE

Study of Serum Endothelin-1 Variation in Sickle Cell Disease: A Systematic Review and Meta-analysis

[1]Chandrakant G Kamble, [2]Prashant Hisalkar, [3]Shekhar B Padhyegurjar, [4]Jagdish D Powar, [5]Santosh E Bidwe
[6]Bhausaheb V Jagadale

ABSTRACT

Introduction: Serum endothelin-1 (ET-1) has been observed in sickle cell disease (SCD). The biological mechanism of the synthesis of ET-1 remains unclear. The aim of the meta-analysis is to provide an overview of serum ET-1 level changes and to discuss the significance of ET-1 in SCD.

Materials and methods: A systematic review and meta-analysis to determine ET-1 level changes during SCD were conducted under Preferred Reporting Items for Systematic Reviews and Meta-Analyses guidelines. Without language restrictions, the articles were identified through BioMed, Embase, PubMed® (US National Library of Medicine, USA), www.bloodjournal.org, PLoS ONE, Web of Science, LILACS (Latin American and Caribbean Health Sciences Literature), BIOSIS Previews, and Elsevier Properties S.A., USA. The web sites that were searched for this study had been published before March 31, 2017. The mean, standard deviation, and sample size of cases and controls were compared and considered for the meta-analysis.

Results: We identified 6 prospective studies out of 309 articles; these are suitable data for the inclusion criteria for this study, of the plasma ET-1 level in SCD. From the included articles, the mean, standard deviation, sample size of cases and controls were compared and calculated by Statistical Package for the Social Sciences (SPSS) software. In all study analyses, $p < 0.005$ was considered to be statistically significant, and the meta-analysis of the study was statistically significant.

Conclusion: The meta-analysis of this study was able to conclude that the plasma ET-1 level in SCD was significantly elevated. This will be helpful to treat the patients and the data could be used for research purposes.

Keywords: Endothelin-1, Meta-analysis, Sickle cell disease.

[1,4-6]Assistant Professor, [2,3]Professor

[1,2]Department of Biochemistry, People's College of Medical Sciences & Research Centre, People's University, Bhopal Madhya Pradesh, India

[3,4]Department of Community Medicine, SMBT Institute of Medical Sciences and Research Centre, Nashik, Maharashtra India

[5]Department of Biochemistry, SMBT Institute of Medical Sciences and Research Centre, Nashik, Maharashtra, India

[6]Department of Physiology, SMBT Institute of Medical Sciences and Research Centre, Nashik, Maharashtra, India

Corresponding Author: Prashant Hisalkar, Professor, Department of Biochemistry, People's College of Medical Sciences & Research Centre, People's University, Bhopal, Madhya Pradesh, India
e-mail: pjhisalkar@yahoo.co.in

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INTRODUCTION

Sickle cell disease is an inherited disorder of hemoglobin caused by replacement of a single nucleotide from thymine to adenine (GTG → GAG), and the amino acid valine is replaced by glutamic acid at the sixth codon of beta-globin chain ($\beta^{s6Glu \rightarrow Val}$).[1] It is a single point mutation responsible for alternation in the properties of the hemoglobin tetramer, with a tendency to polymerize in the deoxygenated state by altering normal, flexible, biconcave-shaped red blood cells (RBCs) into stiff, rigid, sickle cell RBCs.[2] This changes are related to fundamental path physiology, vaso-occlusion, tissue ischemia, sickled erythrocytes, leukocytes, platelets, and endothelial cells.[2,3]

Endothelin-1 is a bipolar peptide that constricts blood vessels found in endothelial cells and also in other cells. The ET-1 and its receptors in neurons have a potential role as neurotransmitter and neuromodulator. The research showed that pain transmission is influenced by ET-1.[4] Lapoumeroulie et al[5] reported that the level of ET-1 rises as there is simultaneous increase in the intensity of pain in SCD patients. However, after treatment with hydroxycarbamide, ET-1 levels decrease with decrease in pain.

Plasma ET-1 production is induced by factors such as proinflammatory cytokines, growth factors, angiotensin-II, mechanical stress, peripheral tissue injury, and hypoxia.[6,7] Earley et al[7] described that plasma ET-1 level is elevated in SCD patients and mice both during and after a vaso-occlusive episode.

Balasa et al[8] reported that increase in plasma homocysteine levels has been shown to be a risk factor for endothelial cell damage and thrombosis, which are implicated in SCD-related vaso-occlusion. Graido-Gonzalez et al[9] have studied and reported that the plasma levels of ET-1, the most potent vasoconstrictor, increased in SCD patients. Circulating ET-1 levels were significantly

elevated in acute pain crisis and remained elevated in the postcrisis period as compared with healthy controls. Phelan et al[10] reported that, the sickle cell disease RBCs which have previously sickling induces the transcription of ET-1 gene fourth fold to eight fold and fourth fold to six fold ET-1 peptide production. In SCD patients, plasma ET-1 levels are increased in steady state and during the crises.[9,11,12] Yanagisawa et al[13] stated that ET derived from vasoconstrictor is elevated in acute chest syndrome with SCD.[14] Patients with hemoglobin S/β-thalassemia had shown elevated ET-1 when compared with control. Pulmonary hypertensive (PH) patients showed increased plasma ET-1 when compared with patients without PH. So he concluded that, plasma ET-1 levels were elevated in patients with PH and SCD.[11]

Telen[15] studied the role of adhesion molecules and vascular endothelium in the pathogenesis of SCD and reported that plasma ET-1 levels had been elevated in patients with SCD. At present, there are no perfect systematic reviews in the exploration that the plasma ET-1 level definitely rises in SCD.

Many more research studies, articles, and reviews have concluded that the plasma ET-1 level is increased in SCD. So the study aim is systematic evaluation and confirmation of the literature and meta-analysis of the plasma level of ET-1 in SCD. This will be the innovation which will help us for advocating and judging the number of hypothesis and results.

MATERIALS AND METHODS

Literature Search Strategy

Meta-analysis is the search of published studies reporting the "Serum Endothelin-1 level variations in Sickle Cell Disease" which was undertaken in accordance with the PRISMA statement for the systematic reviews of interventional studies.[16] A comprehensive search of the literature was conducted in three stages in the following databases: BioMed Central, Amsterdam, PubMed® (US National Library of Medicine, USA), www.bloodjournal.org, PLoS ONE, Web of Science (Thomson Reuters, USA), LILACS (Latin American and Caribbean Health Sciences Literature), Biosis Previews, and Elsevier Properties S.A., USA. The studies published before March 31, 2017 was considered and included in the meta-analysis.

In the first stage, the necessary databases were searched by using the different concerned search criteria. The PubMed® database was searched by using the MeSH® (Medical Subject Headings) term "Sickle Cell Disease" and the keywords "Serum endothelin-1"; the Web of Science® databases were searched using the terms "Sickle Cell Disease" and "endothelin-1." The PLoS ONE® database was searched by simply typing the title name on Google. The blood journals were searched simply by typing the name of the journals, and then the title name of the research topic on the internet. In the above-mentioned journals, published results were very limited to human studies, information/results obtained from books, and editorials; commentaries and conference proceedings were excluded from the studies.

In the second stage, the ideas obtained for reaching the databases using above research criteria were screened by reading the article "TITLE" and "ABSTRACTS." Studies not correlating to topic or not satisfying the inclusion criteria were excluded. Third stage is meant for manuscript screening, and manually searching the data and references for addition. In this stage, articles not satisfying the inclusion criteria were excluded. We excluded literature reviews, cross-sectional studies, studies on animals, articles with missing data, qualitative estimated values of plasma ET-1, and quantitatively estimated values of urine ET-1 in SCD. These inclusion and exclusion search procedures were conducted independently by the reviewer and the final appropriate group of articles was included in the study.

Data Extraction and Analysis

Data Extraction

Data extraction was conducted independently by two authors, using a standardized data extraction form. To resolve the discrepancies that we discussed with the statistician, we extracted the information of the variations of plasma ET-1 level in SCD, on this same title, authors, publication year, and name of study.

Statistical Analysis

The meta-analysis of studies was conducted by examining the variations of plasma ET-1 level in different but related SCDs; statistical analysis was carried out using the latest SPSS software version and Microsoft Excel. For the calculation of Student's t-test, for analysis purpose, we considered the mean, standard deviation, sample size, and number of cases. Statistical graphs were represented using Microsoft Excel software. In all analyses, a p-value < 0.005 was considered to be statistically significant.

RESULTS

We identified 6 prospective studies out of 309 articles; these 6 studies are suitable for inclusion criteria. The screenings and selection of the articles are shown in Fig. 1.

This search was conducted to identify articles up to April 31, 2017.

In the meta-analysis, a total of 109 studies were related articles to be considered (Table 1). A total of 89 studies

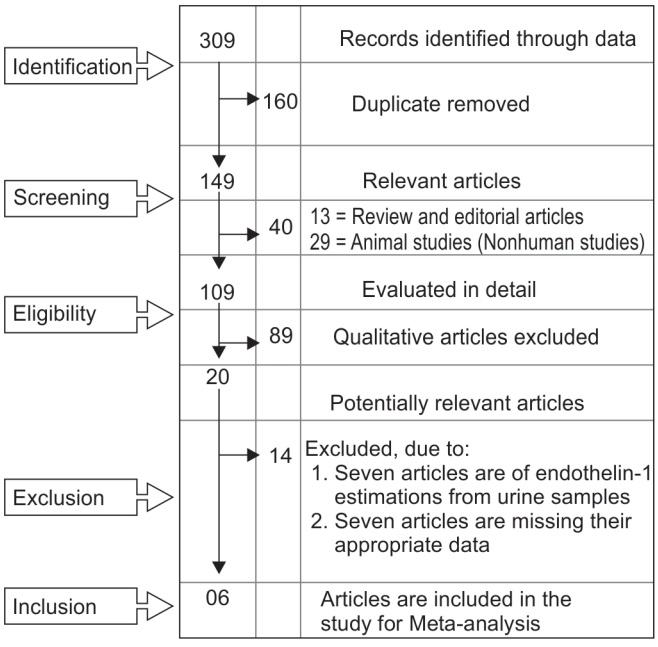


Fig. 1: Selection of studies for meta-analysis

were excluded due to the improper qualitative conclusions (analysis) made by the authors; of these, 33 studies were not able to conclude the (decreases/increase) hypothesis. Only one study (Faller et al 1994) could conclude that the plasma ET-1 level will be constant in the SCD. And two studies showed the decrease in plasma ET-1 in SCD. In 20 studies, plasma and ET-1 level was found to be increased in the SCD samples, but all the data were not reliable for the inclusion. Of 20 studies, 14 were excluded from the meta-analysis due to: (1) Data missing in seven articles and (2) 7 studies that were concerned with the urine ET-1 estimation, which is not related with concerned studies. So, six studies were included in the study.

The six studies that compared serum ET-1 between cases of sickle cell anemia and healthy controls were considered. The independent t-test, pooled standard deviation, and standard errors were calculated by latest SPSS software version (Table 2). Critical values of t from table were compared and the p values were estimated. With the help of t-value and standard error, 95% confidence intervals (CIs) of both cases and controls were calculated.

Quantitative Analysis of Six Studies

The bar diagrams for upper and lower 95% CIs were prepared for testing the possible overlap of these intervals and 95% lower limit for cases was compared to mean of control and 95% upper limit (UL) for controls was compared with mean of cases.

Balasa et al[8] stated that in this particular study the mean of ET-1 is significantly distinct from the control, although CI is slightly overlapped. The UL of controls does not include mean of cases. The calculated t-value is 3.4; DF = 58, and $p < 0.001$, so highly significant.

Thus, it is clearly evident that serum ET-1 is a good marker which can differentiate between the cases and healthy controls of sickle cell anemia.

Graphical Presentation

The statistical graph shows 95% CIs of ET-1 values of cases and healthy controls in six selected studies and one combined study (Graph 1).

Table 1: Selection of studies for meta-analysis in SCD and healthy patient

	ET-1 in SCD cases		*Sample*	*Healthy control*		*Significance of*
Author	*Mean (pg/mL)*	*± SD (pg/mL)*	*size (n)*	*Mean (pg/mL)*	*± SD (pg/mL)*	*the study*
Balasa et al[8]	10.7	2.1	30	8.9	2.0	Increased
Graido-Gonzalez et al[9]	130.9	23.1	13	0.508	6.5	Increased
Ergul et al[17]	11.0	1.4	16	4.2	1.0	Increased
Kenneth et al	0.80	–	7	0.58	–	Not estimable
Nouraie	0.8 (0.4–1.3)	–	28	0.4 (0.1–0.6)	–	Not estimable
Werdehoff et al[23]	10.6 (6.90)	1.9 (1.23)	13	3.0 (1.95)	1.3 (0.82)	Increased
Sowernino-Coker	18.79	–	24	0.58	–	Not estimable
Anne	26.16	–	13	0.52	–	Not estimable
Voskaridou et al	0.94	–	84	0.49	–	Not estimable
Graido-Gonzalez et al[9]	35	11.1	11	0.53	0.50	Increased
Kenneth et al	0.65	–	2	062	–	Not estimable
Kenneth et al	0.65	–	5	0.49	–	Not estimable
Kenneth et al	0.84	–	2	0.67	–	Not estimable
Voskaridou et al	0.67	–	56	–	–	Not estimable
Tharaux et al[22]	0	0	0	0	0	Not estimable
Tharaux et al[22]	0	0	0	0	0	Not estimable
Graido-Gonzalez et al[9]	23.9	9.52	13	0.53	0.50	Increased
Rybicki and Benjamin[11]	0	0	0	0	0	Not estimable
Tharaux et al[22]	0	0	0	0	0	Not estimable
Kenneth et al	0.80	–	7	0.58	–	Not estimable

SD: Standard deviation

Table 2: The t-value, degree of freedom, and level of significance of the cases of sickle cell disease and healthy controls in individual studies, along with combined study

Study	*t-value*	*Degrees of freedom*	*Significance level*
Balasa et al[8]	3.4	58	p < 0.001
Graido-Gonzalez et al[9]	29.77	58	p < 0.0001
Ergul et al[17]	15.82	30	p < 0.000001
Graido-Gonzalez et al[9]	10.29	20	p < 0.001
Graido-Gonzalez et al[9]	20.34	24	p < 0.0001
Werdehoff et al[23]	12.07	24	p < 0.00001
Combined study	38.86	190	p < 0.000001

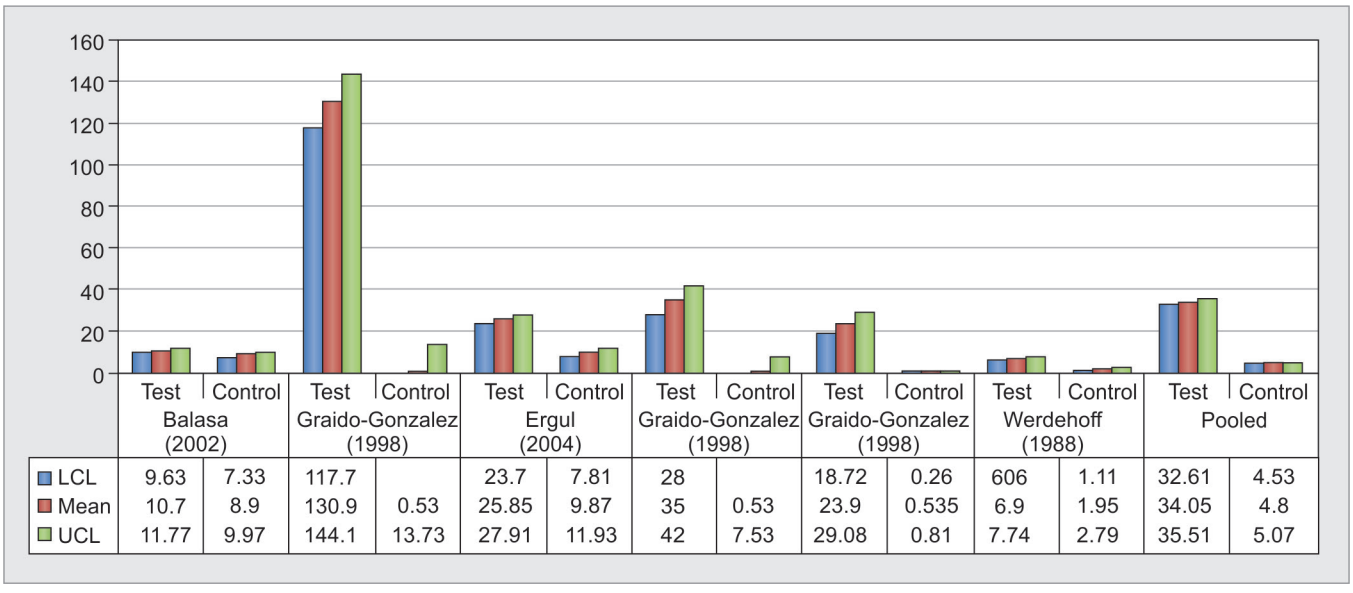
In the study of Balasa et al,[8] the calculated t-value is 3.4 and p shows high significance (p < 0.001). In the bar graph, there is variation; the UL of CI of controls does not include the mean of cases. So, it is statistically highly significant.

Graido-Gonzalez et al[9] showed in a sickle cell study that pooled mean of cases and controls is: 130.9 (95% CI: 117.7–144.1) and pooled mean of healthy controls and CI is 0.53 (UL of 95% CI: 13.73). Interpretation: the bar graph is different from cases and controls. The UL of the control is high and lower limit of the control is below the x axis. Calculated t-value is 29.77, and p is <0.0001, hence, it is statistically significant.

Ergul et al[17] reported that plasma ET-1 levels were significantly increased in (deoxygenated) sickle-shaped erythrocytes and in acute chest syndrome. The pooled mean of cases and controls (Table 3), and their CIs: T = 25.85 (95% CI: 23.7–27.91); C = 9.87 (95% CI: 7.81–11.93); calculated t-value is 15.82; DF 30 and p < 0.000001. So, plasma ET-1 levels were significantly increased.[18]

Graido-Gonzalez et al[9] showed the pooled mean of cases and controls as 35 (95% CI: 28–42) and pooled mean of healthy cases and controls as 0.53 (UL of 95% CI: 7.53). Interpretation: the bar diagram is different from the cases and controls. The UL of control is high and lower limit of the control is going below the x axis. Calculated t-value is 10.29; DF = 20 and p is <0.001, hence, it is statistically significant.

Graido-Gonzalez et al[9] showed that in postcrisis SCD, pooled mean of cases and controls is 23.9 (95% CI: 18.72–29.08) and the pooled mean of healthy controls and CI is 0.53 (UL of 95% CI: 0.26–0.81). Interpretation: the bar graph is different from the cases and controls. The UL of control is high and lower limit of the control is very



	Test	Control	Test	Control	Test	Control	Test	Control	Test	Control	Test	Control	Test	Control
	Balasa (2002)		Graido-Gonzalez (1998)		Ergul (2004)		Graido-Gonzalez (1998)		Graido-Gonzalez (1998)		Werdehoff (1988)		Pooled	
LCL	9.63	7.33	117.7		23.7	7.81	28		18.72	0.26	606	1.11	32.61	4.53
Mean	10.7	8.9	130.9	0.53	25.85	9.87	35	0.53	23.9	0.535	6.9	1.95	34.05	4.8
UCL	11.77	9.97	144.1	13.73	27.91	11.93	42	7.53	29.08	0.81	7.74	2.79	35.51	5.07

Graph 1: Confidence limits of selected studies

Table 3: Serum ET-1 levels in SCD cases and healthy controls with estimation of SMD, 95% CI with LCL–UCL and weighted average of individual and with combined estimated study

Study	*Standard mean difference*	*Lower confidence limit*	*Upper confidence limit*	*Weighted average in percentage (%)*
Balasa et al[8]	1.8	0.73	2.87	31.25
Gradio-Gonzalez et al[9]	130.365	117.099	143.631	13.54
Ergul et al[17]	15.98	13.92	18.04	16.67
Gradio-Gonzalez et al[9]	34.47	27.47	41.47	11.46
Gradio-Gonzalez et al[9]	23.365	17.925	28.805	13.54
Werdehoff et al[23]	9.37	8.15	10.59	13.54
Overall	29.96	27.8	30.72	100

low, calculated t-value is 20.34 and p is <0.0001, hence it is statistically significant. In sickle cell condition oxygen supplementation is low, which stimulates the production and release of ET-1 by vascular endothelial cells.[19]

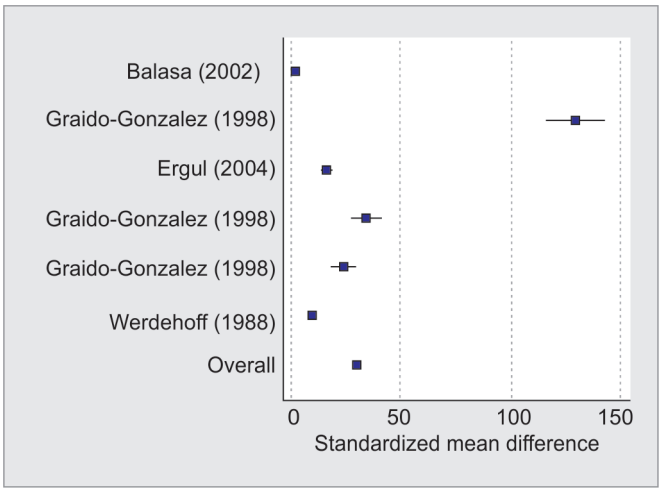
Werdehoff et al[23] showed that there is huge yawning gap in CIs of both cases and controls. The bar graphs also showed huge variation and so the difference is extremely significant. The pooled mean of cases and controls is 6.9 (95% CI: 6.6–7.74) and pooled mean of healthy controls is 1.95 (UL of 95% CI: 2.79–1.11). Interpretation: bar graph showed variation in the cases and controls. The UL of the control is high and lower limit of the control is low. The calculated t-value is 12.07 and p is <0.00001, hence it is statistically significant; the plasma ET-1 level is significantly elevated. Endothelin-1 levels are significantly higher in SCD patients than in controls and were a negative correlation between oxygen saturation and ET-1 levels in SCD patients.[18]

In a combined study, the statistical calculation showed the pooled mean of 96 cases and controls as 35.56 (95% CI: 32.12–34.99) and pooled mean of healthy controls and CI as 4.8 (UL of 95% CI: 4.53–5.07). Interpretation: the bar graph showed the significant variation in the cases and controls. The UL of the control is high and lower limit of the control is low. The calculated t-value is 38.86; DF = 190 and p is <0.00001, hence it is statistically significant, the plasma ET-1 level is significantly elevated in the SCD.

Forest plot (Graph 2) represented the significance of the six important selected articles of the study. In Balasa et al[8] article, the 95% calculated CIs for cases and controls from lower limit to UL is 9.63 to 11.77 and 7.83 to 9.97 respectively; in Graido-Gonzalez et al[9] article, 95% calculated CIs for the cases and controls from lower limit to UL is 117.7 to 144.1 and UL of 95% CI is 13.73; In Ergul et al[17] article, the 95% CIs for the cases and controls from lower limit to UL is 23.7 to 27.91 and 7.81 to 11.93 respectively; in Graido-Gonzalez et al[9] article in SCD crisis study, the 95% calculated CIs for the and controls from lower limit to UL is 28 to 42 an UL of 95% CI is 7.53. Similarly, in Graido-Go et al's[9] postcrisis sickle cell study, the 95% calc CIs for the cases and controls from lower limit t 18.72 to 29.08 and 26 to 0.81 respectively; in Wer et al[23] article, the 95% calculated CIs for the cas controls from lower limit to UL is 6.6 to 7.74 ar to 1.11, respectively, showing huge difference be cases and controls, as compared with all other s In combined study, the 95% calculated CIs for cas controls from lower limit to UL is 32.12 to 34.99 ar to 5.07 respectively; the total random effect in fore shows higher side and significance, so ET-1 level significantly increased in SCD patients.

Graph 2: Forest plo SCD comparable w (overall) study

Forest plot shows that in six independent studies, significant difference in mean of cases and healthy controls is seen. Their standardized mean difference and its 95% CIs show significant difference. The overall estimate shows statistically significant difference in this study. So this forest plot proves conclusively the reliability of serum ET-1 as a biomarker for sickle cell anemia.



Graph 2: Forest plot of 95% CI of serum ET-1 levels in cases of SCD comparable with healthy controls, along with the combined (overall) study

DISCUSSION

The study might be the first comprehensive systematic review and meta-analysis of the serum ET-1 variation in SCD. We have studied and summarized the 6 studies with 192 patients. There is a considerable homogeneity in all articles; hence our data will show the beneficial clinical effects in variations of ET-1 in SCD.

Endothelin-1 produced primarily in the endothelium has important role in vascular homeostasis. Different types of ETs are implicated in several vascular diseases of organs like heart, brain, and general circulation[18,19]; occlusion in microcirculation stimulates the production of cytokines and acute-phase proteins. Cytokines might be involved in pathogenesis of vaso-occlusive mechanisms in SCD, platelet activation, vascular endothelial activation, induction of RBC adhesiveness to vascular endothelium, and ET-1 production.[20]

Balasa et al[8] stated that the plasma ET-1 is highly increased since the calculated t-value is 3.4, DF is 58, and $p < 0.001$, so it is statistically highly significant. Plasma ET-1 and cytokines are significantly increased in SCD and acute vaso-occlusive sickle crisis.[9] *In vitro*, sickle erythrocytes increase ET-1 production by cultured human endothelial cells and ET receptor. An antagonism decreases the vasoconstrictive effects of conditioned media from pulmonary endothelial cells exposed to sickled erythrocytes on aortic rings.[10,12] In Graido-Gonzalez et al[9], the

bar graph is different from the cases and controls. The UL of the control is high and lower limit of the control goes below the x axis. Calculated p is <0.0001, hence it is statistically significant, and the plasma ET-1 level was significantly elevated in SCD.[11] In Ergul et al[17] (fmol/mL values of the article were converted to pg/mL), the statistically calculated p < 0.000001. So, plasma ET-1 levels showed very high and very significantly increased SCD. The ET-1 levels are regulated by endothelial cells, local vasodilators, and vasoconstrictors. In some studies only sickled (deoxygenated) erythrocytes caused a significant increase in ET-1 levels.[17,21]

According to Graido-Gonzalez et al,[9] the UL of the control is high and lower limit of the control is below x axis. Calculated t-value is 10.29; DF = 20 and p is < 0.001, hence it is statistically significant. In another study, ET-1 levels were elevated during sickle cell pain crises and decrease to higher than normal in same patients. Several reports also indicated that there was asymptomatic increase in the levels of ET-1 in SCD.[11,12] Graido-Gonzalez et al[9] mentioned that in post crisis SCD, the ET-1 levels were decreased compared with acute pain crises but more than the healthy controls (0.53). The calculated p is <0.0001, hence it is statistically significant.[11,12] Werdehoff et al[23] indicated that there is huge yawning gap in both CIs of test and controls, so the difference is extremely significant, as we compared with the other studies like Balasa et al,[8] Graido-Gonzalez et al,[9] and Ergul et al.[17] Plasma ET-1 levels were severely increased during the initial stage of acute chest syndrome.[14,18] In some previous SCD studies, it had been reported that the plasma ET-1 is elevated and it is correlated with albuminuria and correlated with its urinary ET-1 level.[22]

The combined study showed that significant variation in the cases and controls. The UL of the control is high and lower limit of the control is low. In combined study, the 95% calculated CIs for the cases and controls from lower limit to UL is 32.12 to 34.99 and 4.53 to 5.07 respectively; weight of study is statistically 100%, the total random effect in the forest plot is highly significant, so the ET-1 is significantly increased in SCD patients.

The calculated t-value is 38.86; DF = 190 and p is <0.00001, hence the study is statistically significant, and the plasma ET-1 level is significantly elevated in the SCDs of all the studies.[9-15,17,18,20,21,23]

CONCLUSION

In this study, this meta-analysis showed that there is extremely significant difference between mean ET-1 of cases and healthy controls. The overall plasma ET-1 estimation showed us the significant difference in the cases and controls. So our hypothesis gets strengthened and strongly reached conclusive position about the reliability of serum ET-1, which could be used as a biomarker for SCD.

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