

Endocan as a Biomarker of Endothelial Function in End Stage Renal Disease

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Abstract

Introduction: Endocan also described as endothelial cell specific molecule (ESM1) is a proteoglycan of 50kda which is highly upregulated in various vascular diseases. Endocan binds CD11a / CD18 integrin on human leukocyte. Pro-inflammatory mediators such as TNFalpha and proangiogenic molecules such as VEGF or FGF 2 stimulate the formation of this mediator. Elevated blood levels of endocan have been reported in patients with cancer (lung, kidney, blood), severe sepsis, and post-transplantation veno-occlusive disease. The purpose of this study is to measure endocan in ESRD patients to demonstrate its relevance to this disease.

Materials and Methods:

Citrated plasma samples were collected from 85 patients who were on maintenance hemodialysis in the dialysis clinic at Loyola University Chicago Hospital. The control group represents 50 normal, drug free individuals. Endocan levels were measured in these plasma samples using a commercially available ELISA method (Lunginnov, Paris, France). VEGF and FGF 2 levels were also measured using commercially available ELISA methods (R&D, Minneapolis, Minnesota).

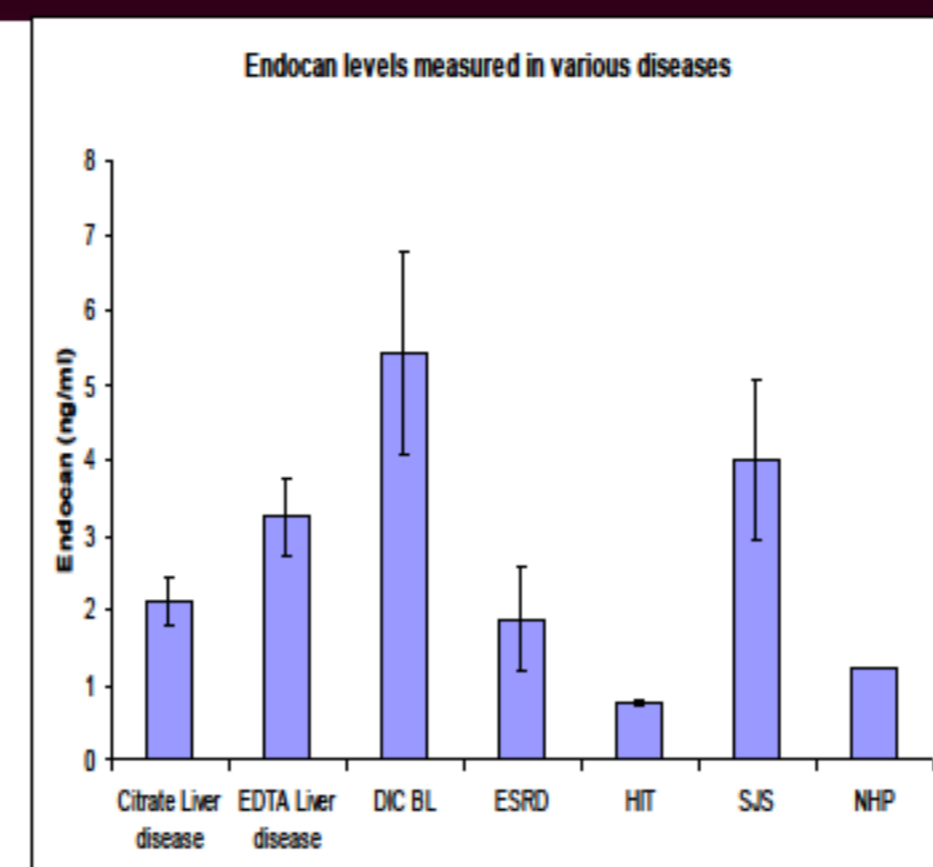
Results:

Endocan levels were found to be significantly higher ($p < 0.05$) in the ESRD patients ($2.6 \pm 1.3 \text{ ng/ml}$) with a wide range ($0.9 + 14.7 \text{ ng/ml}$) in contrast to normal ($1.8 \pm 0.6 \text{ ng/ml}$) with a narrower range of ($1.3 + 3.4 \text{ ng/ml}$). Of the 85 patients, 10 showed greater than 5 ng/ml of this biomarker. Both the VEGF level and FGF 2 levels were also elevated (2-5 folds) in the ESRD group in comparison to the normal. There was a poor correlation ($r < 0.3$) between the elevation of endocan with either VEGF and FGF 2.

Conclusions:

Consistent with earlier reports endocan is elevated in various diseases with endothelial dysfunction. These studies suggest that this biomarker may be a useful prognostic indicator for ESRD. Contrary to the earlier reports the observed poor correlation between endocan and VEGF / FGF 2 may suggest independent regulation of endocan through other mechanisms. This data warrants additional studies to validate the relevance of this marker with the pathogenesis of ESRD.

Introduction



Endocan, or endothelial cell-specific molecule (ESM-1), is a dermatan sulfate proteoglycan expressed by vascular endothelial cells and is detectable in the blood of healthy subjects. Tumor necrosis factor- α (TNF- α) and vascular endothelial growth factor (VEGF) have been shown to induce up-regulation of endocan mRNA, resulting in increased endocan expression. Endocan levels are also increased in several other Pathologic conditions including sepsis/DIC, Steven Johnson's syndrome and related disorders.

Materials & Methods

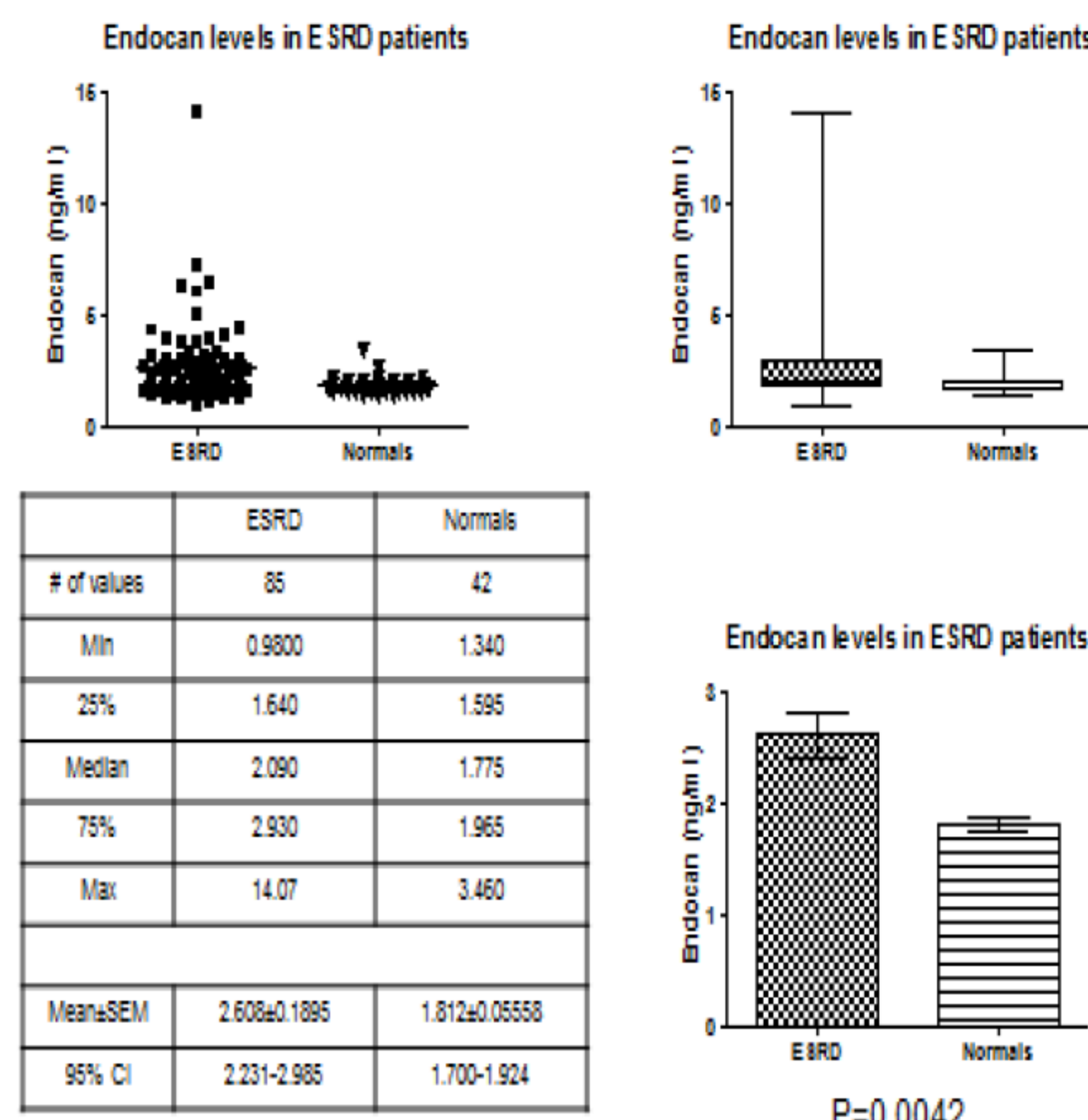
Materials:

This study included 85 ESRD patients undergoing maintenance hemodialysis after appropriate IRB approval and patient consent. Citrated blood samples were collected prior to and immediately after the dialysis session. The blood samples were centrifuged for 15 minutes at 3000g at 4°C and platelet poor plasma (PPP) was extracted. Citrated plasma was frozen at -70°C.

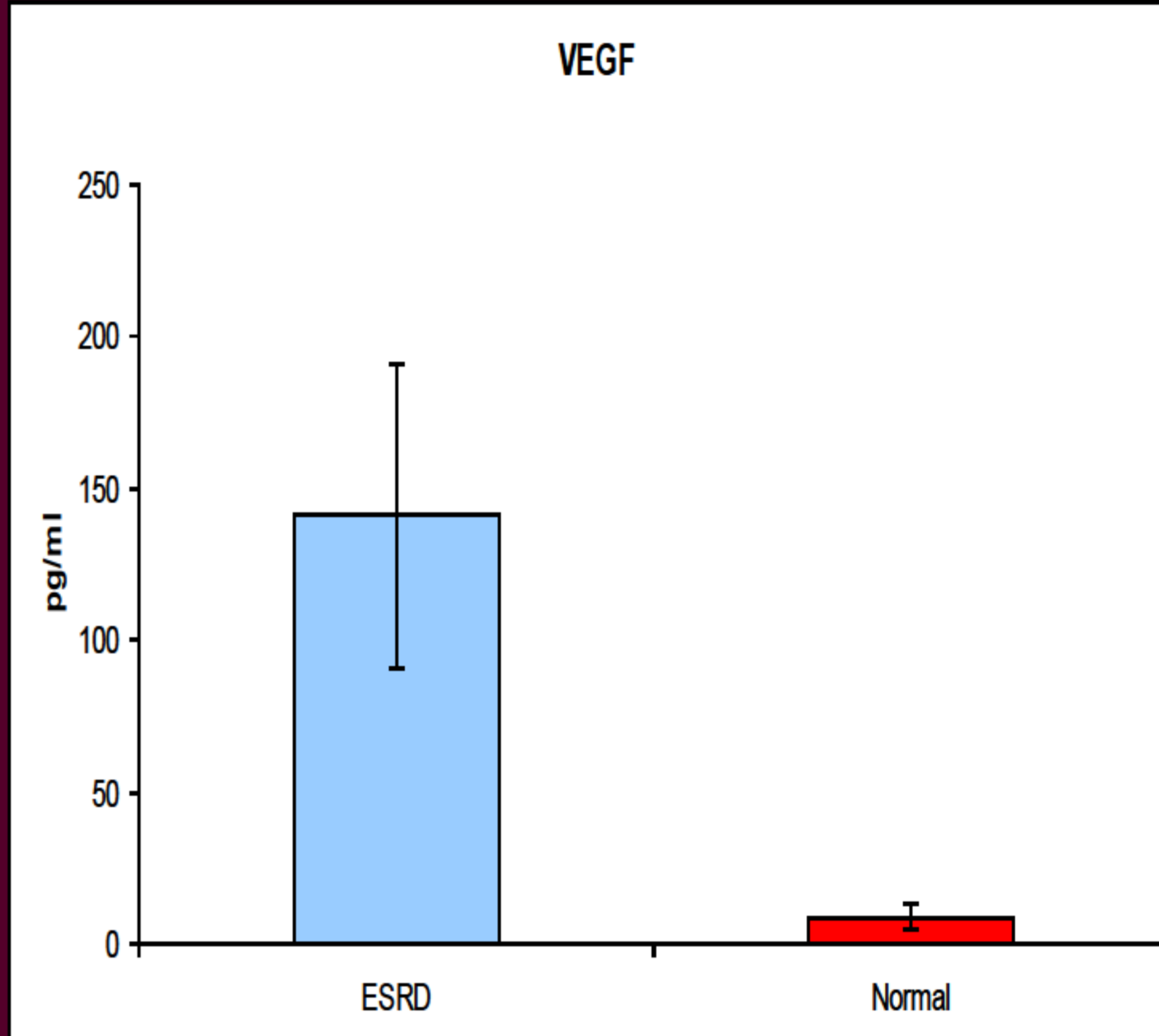
Methods:

Samples collected from ESRD patients were analyzed for the circulating levels of endocan using a commercially available ELISA method (Lunginnov, Paris, France). FGF-23 was measured by using a sandwich ELISA kit (Millipore, St. Charles, Missouri). The VEGF analysis was carried out using Quantikine sandwich ELISA method (R&D Systems, Minneapolis, Minnesota). Plasma samples collected from normal male and female (n=42-80) comprised the normal group.

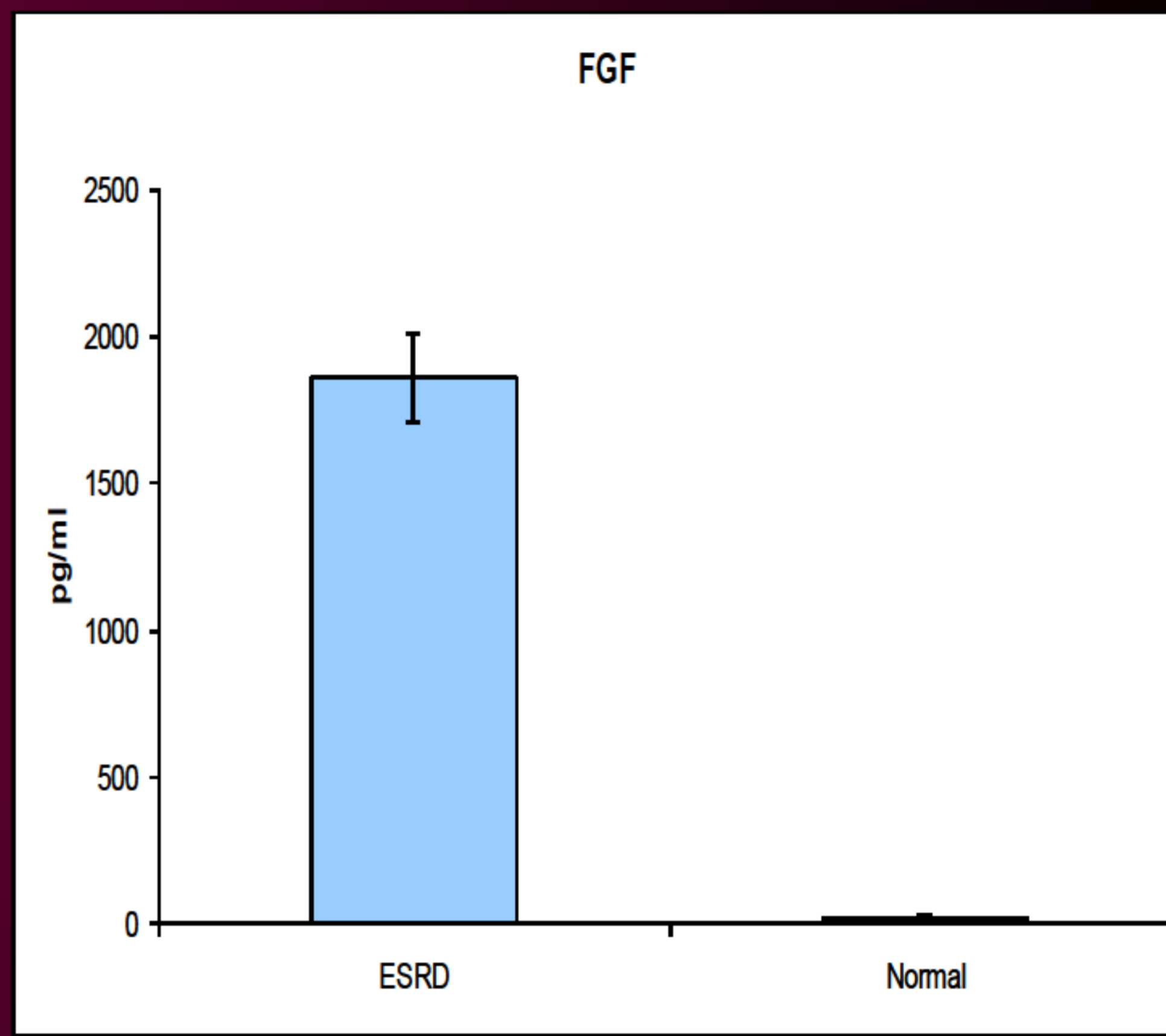
Endocan Levels in ESRD



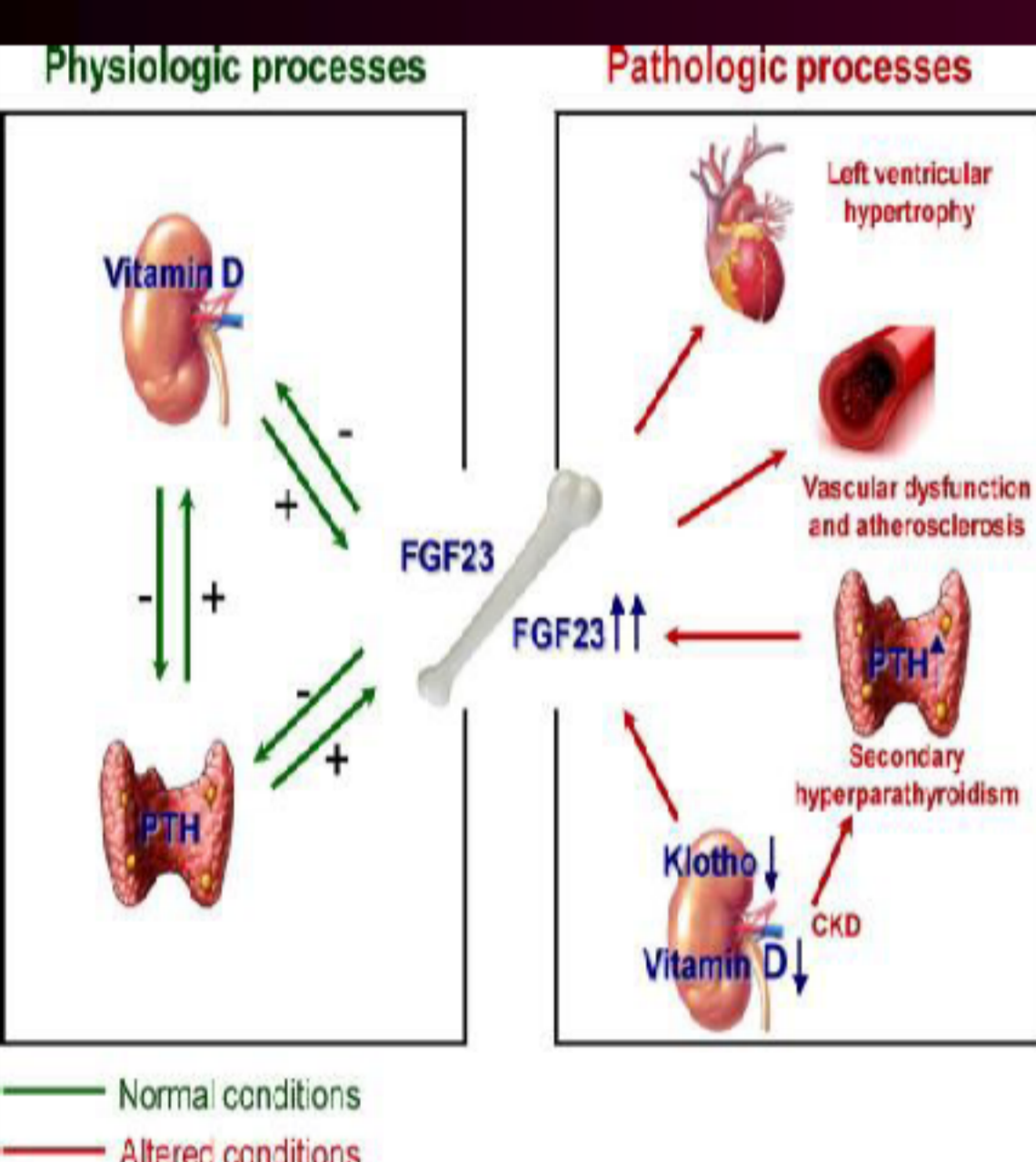
VEGF (pg/ml) ESRD vs Normal



FGF-23 (pg/ml) ESRD vs Normal

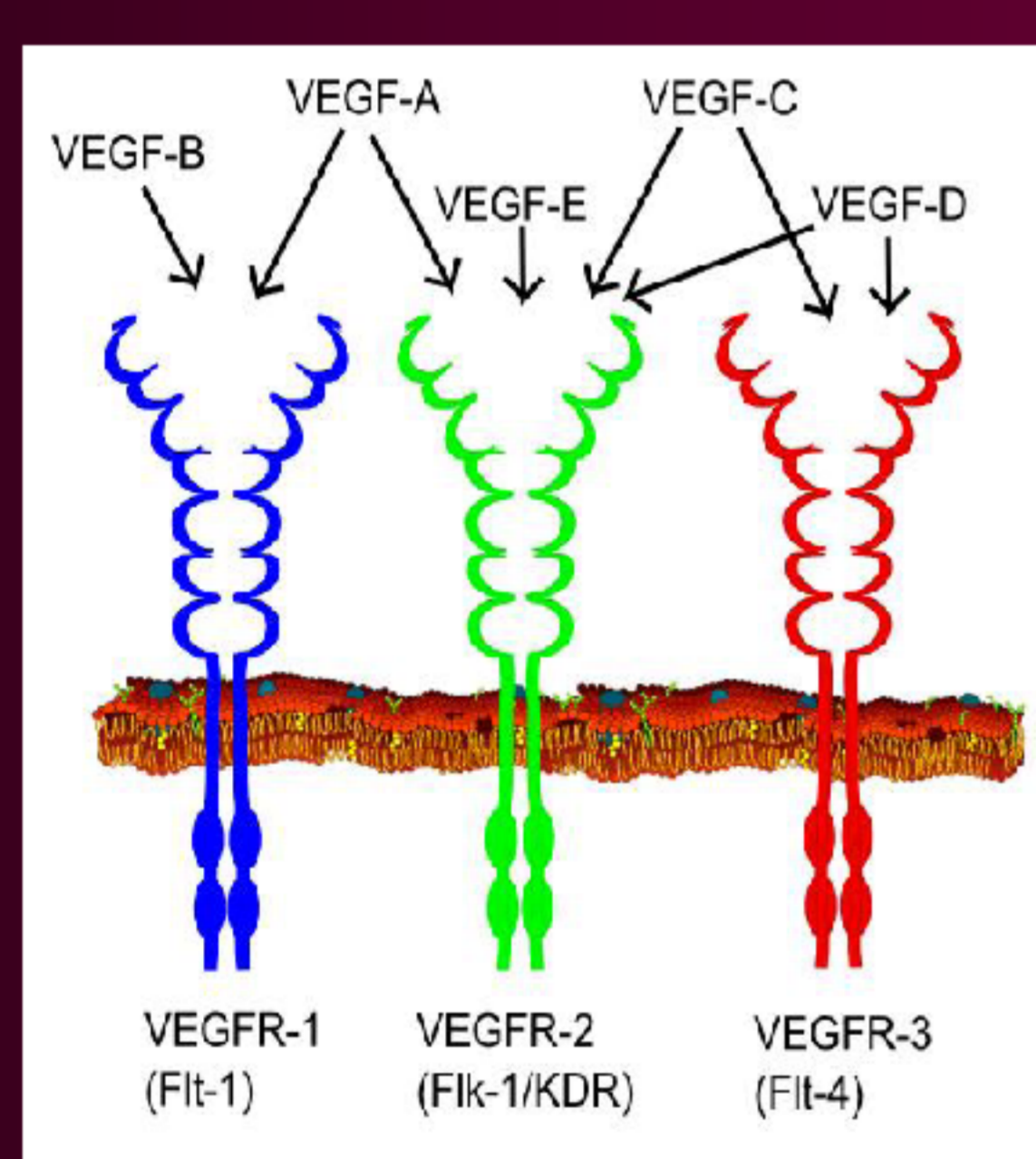


FGF-23



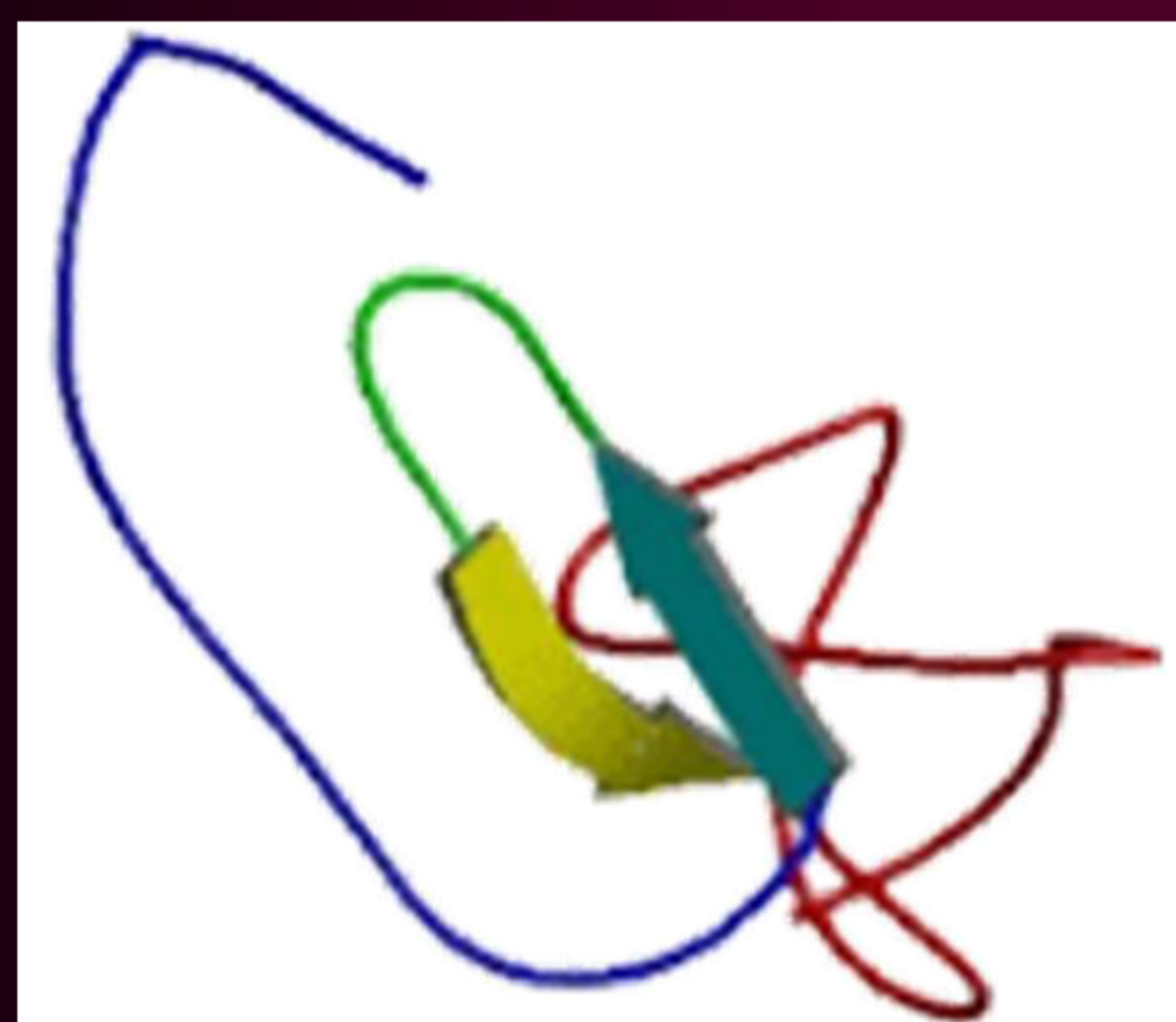
Fibroblast growth factor 23 (FGF-23) is a protein in the heparin binding growth factor FGF family responsible for phosphate metabolism. Altered levels of FGF-23 are associated with various pathologic conditions.

VEGF



Vascular endothelial growth factor (VEGF) is a signal protein produced by vasculogenesis and angiogenesis stimulating cells. VEGF is a potent mediator of these processes in various diseases.

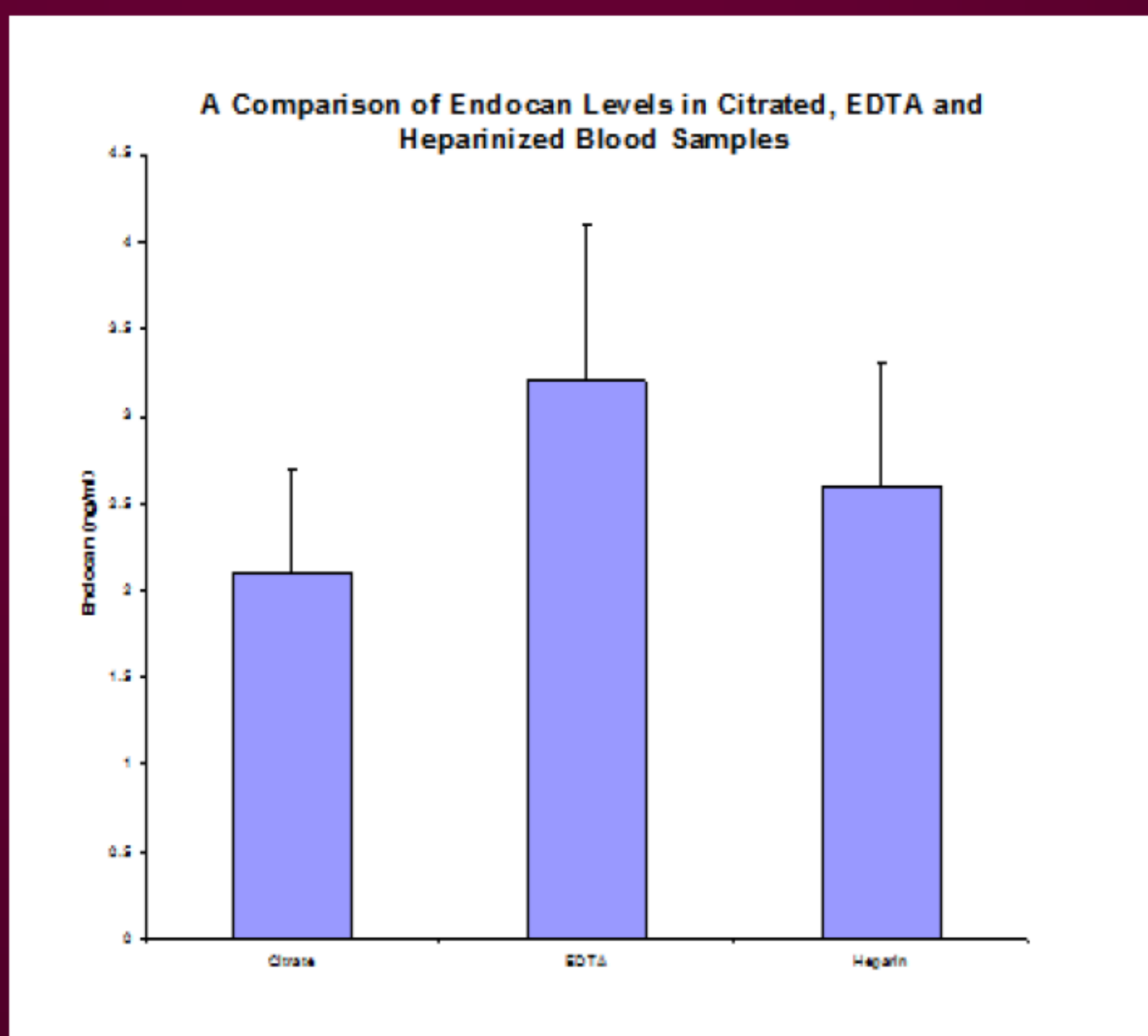
Endocan: New Biomarker of Endothelial Dysfunction



Endocan, previously called endothelial cell specific molecule-1, is a soluble proteoglycan of 50 kDa, constituted of a mature polypeptide of 165 amino acids and a single dermatan sulphate chain covalently linked to the serine residue at position 137. This dermatan sulphate proteoglycan, which is expressed by the vascular endothelium, has been found freely circulating in the bloodstream of healthy subjects. In pathologic conditions such as ESRD, endocan levels are elevated.

Results

Endocan Levels in Normals



Summary

1. ESRD represents a complex syndrome in which endothelial dysfunction contributes to the pathogenesis by mediating inflammatory responses.
2. Endocan represents a novel biomarker of endothelial function which has been shown to be elevated in several diseases. In this study endocan levels were widely dispersed with many patients with high levels of this biomarker.
3. The FGF-23 levels ranged from 0 to 5934 pg/ml (Mean = 1861 pg/ml with S.E.M = 151) in contrast to the normal levels of $18.4 \pm 6.1 \text{ pg/ml}$. Of the 85 patients, 67 (56.3%) had levels greater than 1000 pg/ml FGF-23 levels.
4. The VEGF levels ranged from 8.2 to 3673 pg/ml (Mean = 141 pg/ml with S.E.M = 50.1) in contrast to the normal levels of $8.7 \pm 4.2 \text{ pg/ml}$.
5. Although endocan levels were elevated in the ESRD patients, there was no correlation between circulating endocan levels and VEGF/FGF levels.

Conclusions

End Stage Renal Disease represents a complex polypathologic disorder where endocan and such growth factors as VEGF and FGF-23 may play an important role. Endocan and these growth factors are elevated in ESRD patients and this may contribute to the cardiovascular and cerebrovascular events observed in ESRD through multiple mechanisms at target organs.

Clinical Implications

Endocan along with FGF 23 and VEGF are found to be increased in ESRD patients. These patients also exhibit wide variations in the levels of these biomarkers. There is no correlation between the concentration of endocan and these two growth factors. Besides their regulatory functions endocan and both of these growth factors may be useful as prognostic variables which can be used for risk stratification and clinical management of ESRD patients.