Increased Expression Of Fractalkine (CX3CL1) As Possible Role In In Active Systemic Lupus Erythematosus(SLE)

Sahar S. Zalam[1], Howida M. Sharaf[2], Mervat Mohie Eldin Ali[1]and Samia Taher[3]

Clinical Pathology[1], Internal Medicine Departments[3], Al-Azhar University Faculty of Medicine for Girls and Clinical Pathology[2], king Khalid university- KSA

Thelittleruby@Hotmail.Com

Abstract: Background: Fractalkine (CX3CL1), the unique member of the cx3c chemokine subfamily, in endothelial-related inflammation.Objective.based on the function of fractalkine (cx3cl1), the unique member of the cx3c chemokine subfamily, in endothelial-related inflammation, we hypothesized a role for cx3cl1 in sle and its relationship with disease activity and renal impairment.Method: Fkn /CX3CL1 Expression Was Studied In 20 Female Patients With SLEDivided Into Active And Inactive According To SLE Disease Activity Index (SLEDAI) & 10 Healthy Volunteers As A Control Group, Using Quantitative Real-Time Polymerase Chain Reaction (RT-PCR) At Al-Zahraa &Ain Shams University Hospitals. Results: There was A Highly Significant DifferenceBetween Fkn/CX3CL1 Mrna Expression In Active SLE Patients And Inactive SLE Patients And Control Group, While There Were No Significant Differences WasObserved Between InactiveSLE Patients And Control Group. There Was A Highly Significant Increase Of Serum Sfkn Levels In ActiveSLE Patients As Compared To Inactive Patients And Control Group, Also A Significant Increase In Sfkn Levels Was Found In Inactive Patients When Compared To Control Group. There Were A Significant Positive Correlations Between The Fkn /CX3CL1 Mrna Expression & Serum Sfkn Levels In Active SLE Patients AndSLE Disease Activity Index (SLEDAI),Dsdna, Urea And Createnin. In Conclusion, We Have Demonstrated The Enhanced Expression Of Fkn/CX3CL1In Patients With Active SLE. OurResults Suggest That FKN/CX3CL1Plays A Part In The Disease Processe Activity, Including Inflammation And Vascular Injury. However,Further Studies Will Be Needed To Determine The Relative Importance Of FKN/CX3CL1Compared With Other Chemokines In SLE,And To Clarify The Specific Role Of FKN/CX3CL1In SLEWhich Is Distinct From Its Role In Other Inflammatory Diseases.

[Sahar S. Zalam, Howida M. Sharaf, Mervat Mohie Eldin Aliand Samia Taher.**Increased Expression Of Fractalkine (CX3CL1) As Possible Role** In **In Active Systemic Lupus Erythematosus(SLE).** *J Am Sci*2013;9(11):66-71]. (ISSN: 1545-1003). http://www.jofamericanscience.org. 11

Key Words:SLE**,**Fractalkine, CX3CL1, RT-PCR

1. Introduction:

Systemic Lupus Erythematosus (SLE) Is An Autoimmune Disease Characterized By Multiorgan Damage With Infiltration And Sequestration Of Various Leukocyte Subpopulations, And By The Presence Of Autoantibodies (Ruiz-Irastoza *et al*., 2001). It's Etiology Is Known to Involve Dysregulation of the Immune System, Leading To A Functional Imbalance of T cell Subsets, Production Of A Wide Range Of Autoantibodies, And Polyclonal B CellActivation. In Addition, the Importance of Dysregulation of Cytokine Expression Has Been Noted (Dean *et al.*, 2000).

The Role Of Vascular Injury In The Pathogenesis Of SLE Has Been Well Described. Circulating Immune Complexes of Autoantibodies and Self-Antigens Are Deposited In the Vascular Walls Of SLE Patients And Activate

The Complement Pathways. The Resulting Complement Products Stimulate Leucocytes To Injure The Vascular Endothelium, Leading To Blood Vessel Destruction And Organ Injury (E.G. Glomerulonephritis And Vasculitis) (Hoet al., 2003 and Yoshimoto e t al., 2007)

The Membrane-Spanning Protein Fractalkine (Fkn) Also Known As CX3CL1or Neurotactin Is A Member Of The CX3C Family Of Chemokines And Contains A Conserved CX3C Domain Atop A Mucin-Like Stalk In Its Extracellularregion .Fkn Is Expressed At Very Low Levels By Resting Endothelial Cells But Undergoes Marked Upregulation Following Of The Cells By Cytokines Such As Tumor Necrosis Factor (TNF) And Interleukin-1(IL-1). Fkn Is Also Known To Function As A Cellular Adhesion Molecule And To Attract Cells Expressing Its Receptor, CX3CR1 (Umehara *et al*., 2001 and Tadros etal., 2003).

Studies Of Vasculitis (Lucas *et al.*, 2001and Cockwell *et al.*,2002) Have Shown Endothelial Cells (Ecs) To Be The Major Cellular Source Of CX3CL1, And EC-Derived CX3CL1 Likely Plays A Key Role In Such Pathological Conditions As Vascular Inflammation, Glomerulonephritis And Pulmonary Arterial Hypertension (Umehara *et al.*,2004).

Thus,CX3CL1 Appears To Possess Immunoregulatory Properties That Affect Inflammatory And Immune Cell-ECInteractions And Inflammatory Responses At Inflamed Sites. Indeed, Investigations By Several Groups, Have Implicated CX3CL1 In A Variety Of Inflammatory Disorders, Including Glomerulonephritis, RA, Systemic Sclerosis.And SLE (Hasegawa *et al.,* 2005 And Yajima *et al.*,2005).

2. Subjects andMethods:

This Study Was Carried On Twenty Female Patients With SLE, Attend The Out-Patient Clinics And Those Admitted To Internal Medicine Department Of Al-Zahraa &Ain Shams University Hospital.

- All Patients Fulfilled The American Rheumatology Association (ARA) Criteria For Diagnosis Of SLE (Tan *et al.*,1982).
- SLE Patients Were Classified Into Two Groups, Active And Inactive According To The SLE Disease ActivityIndex (SLEDAI) (Bombardier Et Al., 1992):

1- Group I Included 10 Female Patients With Active SLE, Their Ages Ranged From 17and 34 Years Old With A Mean Of 23.8±5.31

2- Group II Included 10 Patients With Inactive SLE, Their Ages Ranged From 17 To 52 Years Old With A Mean Of 26.16±9.6. In Addition To10 Healthy Volunteers As A Control Group.

All Patients And Controls Were Subjected To The Following:

1- Thorough History And Clinical Examination.

2- Laboratory Investigations: Each Blood Sample Was Divided Into Two

-CBC Using Fully Automated Cell Counter, And ESR.

- Kidney Function Tests Were Done Using Hitachi 911 Auto-Analyzer.
- C-Reactive Protein (CRP), Was Carried Out Using Biocientifica S.A Kit.

Positive Test Showing A CRP Level ≥ Than 8 Mg/L (Tillet And Francis, 1930).

- Anti-Double Stranded DNA (Ds DNA) Antibody Detected By Indirect Immunofluorescence Method Using Kit Suuplied By Biosystem S.A. Costa Brava 30, Barcelona (Spain).
- Soluble Fractalkine (Sfkn) Was Detected By ELISA Kits From R&D System, Minneapolis-MN, USA;
- Expression ofFkn/ CX3CL1 Mrna byReal -Time RT PCR:

A- Isolation and Purification of Total RNA:

Total RNAExtracted From Pbmcs According Protocol Of Rneasy Total RNA Reagent Set (Qiagen, Germany). The Concentration of RNA Was Determined Spectophotometrically by the Absorbance at 260nm.One Microgram of RNA Was Incubated For 15 Min. At Room Tempera Ture With Dnase I (1 U/Ug, Promga,USA), Followed By Thermal Inactivation Of The Enzyme(65°C For 10 Min.) And A Rapid Cooling Down To 4C. The Purity Of RNA Was Estimated By The Ratio Of The Absorbance At 260/280

B- Cdna Synthesis:

The Reverse Transcription Reaction Was Carried Out In Total Volume Of 20 Ul Of 1X Reverse Transcriptase Buffer Containing 10mm Dithiothreitol, 500 Um Deoxynucleotide Triphosphate., 3um Oligo (Dt), 10 Units Of Rnasin And 2 Units Of Avian Myeloblastosis Virus (AMV) Reverse Transcriptase To This Mixture 1ug Of Total RNA Treated With Dnase I Was Added. The Reaction Was Allowed To Proceed For 60 Min. At 42 ^{o}C, Followed By 5 Min. Of Heating At 95°C And Rapid Cooling On Ice. The Cdna Was Stored At -20C Until Use. All Reagents For RT-PCR Were Supplied By Promega, USA.

C-Quantitative Real- Time PCR And Continual Monitoring Of PCR Product:

The Primers Used In The PCR Were As Follows:

For Fkn,

5'GCTGAGGAACCCATCCAT (Sense)

And 5'GAGGCTCTGGTAGGTGAACA(Antisense) (Yajima *et al.*,2005).

We Also Quantified Transcripts Of The B Actin Gene As Endogenous RNA Control And Each Sample Was Normalized On The Basis Of Its B Actin Content . We Selected The B Actin Gene As An Endogenous Control Because The Prevalence Of Its Transcripts Is Similar To That Of The Target Gene. The Oligonucleotide Primer Sequence Of B Actin Gene Was:

5'CCCAAGGCCAACCGCGAGAAGAT (Sense)

And 5' GTCCCGGCCAGCCAGGTCCAG (Antisense) (Yajima Et Al., 2005).

Calibration Curve Consturuction:

Separate Calibration Curves For B Actin And Fkn Were Constructed With Fourfold Serial Dilution Of Control Cdna. All Calculated are Relativeto This Concentration.

PCR Amplification:

PCR Reaction Was Monitored In Light Cycler System (Roch Molecular System, France) Using L-C Fast Start Reaction Mix SYBR Green I (Roch Diagnostic)(Wittwer *et al.*,1997). Thermo Cycling Was Done In A Final Volume Of 10 Ul Containing 1.5 Ul Of Cdna Sample Or Calibrator; 3mm Mgcl ; 0.5 Um Of Fkn Primers ; 0.3 Um Of The B Actin Primers ; 1ul Of LC Fast Start Reaction Mix SYBR Green I / Enzyme (Including Taq Dnapolymerase, Reaction Buffer And Deoxynucleotide Triphosphate Mixture) . After The Reaction Mixture Was Loaded Into The Glass Capillary Tube; The Cycling

Conditions Were To As Follows: Initial Denaturation At 94°C For 10 Min. To Denature the Cdna and to activate theTaq DNA polymerase, followed by 45 Cycles of Denaturation At 94°C For 15 Sec., Annealing At 60°C For 5 Sec. and extension At 72 °C forB Actinfor 18 Sec.

After PCR, A Melting Curve Was Constructed by Increasing The Temperature From 65 °C With A Temperature Transition Rate of 0.1°C/S. The Assay wascompleted In 1hour. The Light Cycler Apparatus Measured The Fluorescence Of Each Sample In Every Cycle At The End Of The Annealing Step. The Second Derivative Maximum Method Was Used To Determine The Crossing Point (Cp) Automatically For The Individual Samples. This Was Achieved By A Software Algorithm (Ver.3.5) That Identifies The First Turning Point Of The Flurescence Curve Corresponding To The First Maximum Of Second Derivative Curve, Which Serves As The Cp. The Light Cycler Software Constructed The Calibration Curve By Plotting TheCp Vs The Logarithm Of The Concentration Of Each Calibrator. The Concentration In Unknown Samples Were Calculated By Comparing Their Cps With The Calibration Curve. To Correct For Differences In Both RNA Quality And Quantity Between Samples, Data Were Normalized Using The Ratio Of The Target Cdna Concentration To That Of B Actin

Statistical Analysis:

Data Was Analyzed Using The Computer SPSS Version 12. An Optimal Cutoff PointOf FknGene Expression Was Defined By X +2SD. Parametric Data Was Expressed As Mean ± SD And Nonparametric Data Was Expressed As Number And Percentage. Student's T Test Was Done to Compare between Groups. Pearson Correlation Coefficient Was Done To Correlate Between Different Parameters Among Groups. *P*Value <0.05 Was Considered Non Significant, *P* Value Of >Was 0.05 Considered Significant, *P* Value Of < 0.01 Was Considered Highly Significant.

3. Results

The Results and Data Were Collected and Analyzed In Tables (1 - 6) andFigs. (1 and 2). Table (1) Shows Highly Significant Increase In Sfkn Levels In Active When Compared To Inactive SLE Patients And Control Group,While There Is A Significant Increase In Sfkn Levels In Inactive SLE Patients When Compared To Control Group.

The Results Were Tabulated From Tables1 - 6 .

Table 1: Comparison of Sfkn Levels among All Studied Groups.

Variable	**Controls (N=10)) Mean ± SD**	**Inactive SLE (N=10) Mean ± SD**	**Active SLE (N=10) Mean ± SD**	***P*- Value**
Sfkn(Pg/ Ml)	70.5±6.85	282±251.73	1003.5±401.37	*P*1 0.001** *P*2 0.001** *P*3 0.05

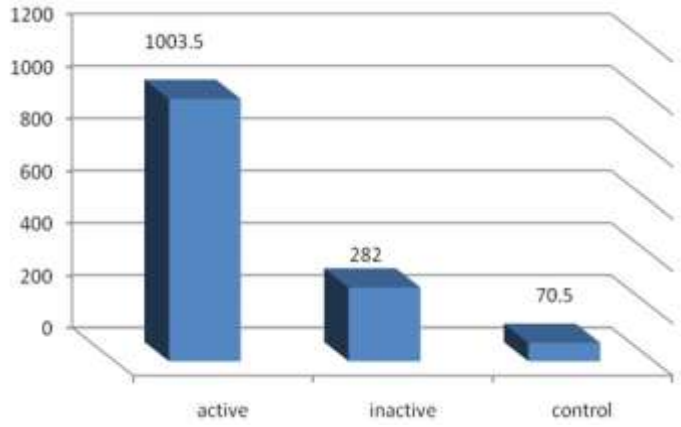

Fig. 1: Sfkn Levels between All Studied Groups.

Table 2: Expression Of Fkn /CX3CL1 GeneIn SLE Patients And Control Group.

Variable	Controls (N=10)) Mean ± SD	Inactive SLE (N=10) Mean ± SD	Active SLE (N=10) Mean ± SD	*P*- Value
Fkn /CX3CL1	0.23±0.036	0.26±0.04	0.57±0.20	*P*1< 0.000** *P*2 0.001**

Table 3: Comparison Of Kidney Functions Between All Studied Groups.

Variables	Controls (N=10)) Mean ± SD	Inactive SLE (N=10) Mean ± SD	Active SLE (N=10) Mean ± SD	*P*- Value
Urea(Ur) (Mg/Dl)	23±3.30	28.5±4.01	28.5±4.01	*P*1< 0.01** *P*2< 0.01** *P*3< 0.01**
Createnin(Cr) (Mg/Dl)	0.65±0.18	0.9±0.31	1.94±0.46	P1< 0.01** P2< 0.01** P3 < 0.05*

Table 4: Correlation Between Sfkn By ELISA And Studied Variables In Active SLE Group.

Variables	R	*P*Value	Significance
SLEDAI	**0.932**	< 0.01	**HS**
Dsdna	**0.882**	**< 0.01**	**HS**
Rbcs $\times 10^{6}$ /Cmm	**0.239**	**> 0.05**	**NS**
Wbcs $\times 10^{3}$ /Cmm	**0.149**	**> 0.05**	**NS**
PLT $\times 10^{3}$ /Cmm	**-0.073**	**> 0.05**	**NS**
ESR (Mm/Hr)	**0.686**	**< 0.05**	**S**
CRP	**0.750**	**< 0.05**	**S**
Ur (Mg/Dl)	**0.878**	**< 0.01**	**HS**
Cr (Mg/Dl)	**0.939**	**< 0.01**	**HS**

.

Table 5: Correlation Between Fkn /CX3CL1Mrna Expression By PCR And Studied Variables In Active SLE Group

Variables	R	*P*Value	Significance
SLEDAI	**0.756**	< 0.01	**HS**
Dsdna	**0.693**	**< 0.01**	**HS**
Rbcs $\times 10^{6}$ /Cmm	**0.457**	**> 0.05**	**NS**
Wbcs $\times 10^{3}$ /Cmm	**0.137**	**> 0.05**	**NS**
PLT $\times 10^{3}$ /Cmm	**-0.176**	**> 0.05**	**NS**
ESR (Mm/Hr)	**0.619**	**< 0.05**	**S**
CRP	**0.561**	**< 0.05**	**S**
Ur (Mg/Dl)	**0.812**	**< 0.01**	**HS**
Cr (Mg/Dl)	**0.879**	**< 0.01**	**HS**

Table 6: Correlation Between Sfkn By ELISA And Studied Variables In Inactive SLE Group

Variables	R	*P*Value	Significance
Dsdna	**-0.272**	**> 0.05**	**NS**
Rbcs $\times 10^{6}$ /Cmm	**-0.375**	**> 0.05**	**NS**
Wbcs $\times 10^{3}$ /Cmm	**-0.338**	**> 0.05**	**NS**
PLT $\times 10^{3}$ /Cmm	**-0.458**	**> 0.05**	**NS**
ESR (Mm/Hr)	**0.175**	**> 0.05**	**NS**
CRP	**-0.337**	**> 0.05**	**NS**
Ur (Mg/Dl)	**-0.215**	**> 0.05**	**NS**
Cr (Mg/Dl)	**-0.249**	**> 0.05**	**NS**

3. Discussion:

Systemic Lupus Erythematosus (SLE) Is A Rheumatic Inflammatory/ Autoimmune Disease In Which Innate And Adaptive Immune Response Are Involved. In Consequence Different Mechanisms Immune/ Inflammatory

Participate In Endothelial Damage And Coagulation Pathways Activation. Although The

Exact Cause Of SLERemains Elusive, Increasing Evidence Indicates Vascular Injury As A Major Factor In SLE Pathogenesis (Pizarro*et al.*, 2007).

Chemokines And Their Receptors Are Closely Involved In Regulating Organ-Specific Leukocyte Trafficking And Inflammation,Suggesting They Have Important Roles In The Pathophysiology Of Autoimmune Diseases Such AsSystemic Lupus Erythematosus (SLE), Rheumatoid Arthritis And Systemic Sclerosis.(Ferreira *et al.*,2010) Some Proin-Flammatory Cytokines May Directly Stimulate Angiogenesis Or Act Indirectly By Enhancing The Production Of Angiogenic Chemokines(Brian Jones *et al.*,2012).

In Contrast To Other Chemokines, Fkn /CX3CL1 Exists In Two Forms, Each Mediating Distinct Biological Actions. The Membrane-Anchored Protein,Which Is Primarily Expressed On The Inflamed Endothelium, Serves As An Adhesion Protein Promoting The Retention Of Monocytes And T Cells In Inflamed Tissue (Bjerkeli *et al.*,2007).

The Soluble Form Resembles More A Conventional Chemokine And Strongly Induces Chemotaxis. Both Chemotaxis And Adhesion Are Mediated By The G Protein-Coupled Receptor CX3CR1. Based On These Chemotactic

And Adhesive Properties, CX3CL1 Has Been Thought To Play An Important Role In Inflammation, And Indeed, Accumulating Evidence Indicates That CX3CL1/CX3CR1 Are Involved In The Pathogenesis Of Various Inflammatory Disorders Such As Glomerulonephritis, Rheumatoid Arthritis And Systemic Lupus Erythematosus (SLE) (Bjerkeli *et al.*,2007).

Based On The Function Of Fkn /CX3CL1, The Unique Member Of The CX3C Chemokine Subfamily, In Endothelial-Related Inflammation, We Hypothesized A Role For Fkn /CX3CL1 In SLE And Its Relationship With Disease Activity And Renal Impairment.

The Present Study Show Highly Significant Increase In Sfkn Levels In Active SLE Patients When Compared To Inactive Patients And Control Group, While There Is A Significant Increase In Sfkn Levels In Inactive Patients When Compared To Control Group. This Findings Were In Agreement Of Yajima *et al.*(2005) Who Found That Serum Levels Of Sfkn Were Significantly Higher In Patients With SLE (Mean ±SEM 452.7 ± 118.0 Pg/Ml) Than In Healthy Controls (Mean ± SEM 3.2 ±3.2 Pg/Ml;(P<0.01).

Also, Our Findings Were In Coincidence With Sato Et Al. (2006), Who Reported That Serum Levels Of Soluble CX3CL1 In Patients With SLE (402.28 Pg/Ml) Were Higher Than Those In Patients With Other Autoimmune Disorders.

This Study Show Highly Significant Increase In Fkn /CX3CL1 Mrna Expression In Active SLEPatients When Compared To Inactive SLE Patients And Control Group, While There Is No Significant Differences In Fkn /CX3CL1 Mrna Expression In Inactive SLE Patients As Compared To Control Group. On The Other Hand Yajima *et al.*(2005) Found That Fkn/CX3CL1Expression In Peripheral Blood Mononuclear Cells (Pbmcs) From Active SLE And Control Group Was Markedly Weak, And No Significant Difference Between Two Groups Was Observed. One Possible Explanation For The Apparent Inconsistency Between These Studies Is That Ethnic Specific Genetic Variation Could Greatly Influence Host Immunity . Another Possible Explanation Might Be The Relatively Small Size Studied Population.

Furthermore ,There Was A Significant Positive Correlation Between Sfkn And SLE Disease Activity Index (SLEDAI) And Dsdna. Our Finding Were In Coincidence Of Yajima *et al.*(2005), Who Observed That Serum Levels Of Sfkn Were Positively Correlated With Disease Activity As Measured By The SLEDAI (R =0.351,P<0.05) And Were Also Positively Correlated With Anti–Double-Stranded DNA (Anti-Dsdna) Antibody Titers (R=0.300, P <0.05).

As Regard Kidney Function Tests, There Were A Highly Significant Increase In Ur And Cr Levels In Active SLE Patients When Compared To Inactive Patients And Control Group. Also, There Was A Significant Increase In Ur And Cr Levels In Inactive SLE Patients When Compared To Control Group. Our Results Were In Accordance With Ho *et al.*(2003), Who Stated That, Elevated Serum Creatinine And Urea Concentrations Are Commonly Used For The Assessment Of Impaired Glomerular Function, Which May Be Caused By Circulating Nephritogenic Autoantibodies And Immune Complexes.

In Conclusion, We Have Demonstrated The Enhanced Expression Of Fkn /CX3CL1 In Patients With Active SLE. Our Results Suggest That FKN/CX3CL1 Plays A Part In The Disease Process Activity, Including Inflammation And Vascular Injury. However, Further Studies Will Be Needed To Determine The Relative Importance Of FKN/CX3CL1 Compared With Other Chemokines In SLE, And To Clarify The Specific Role Of FKN/CX3CL1 In SLE Which Is Distinct From Its Role In Other Inflammatory Diseases

References

1. Bazan, J.F., K.B. Bacon, G. Hardiman, W. Wang, K. Soo, D. Rossi, D.R. Greaves, A. Zlotnik And

T.J.Schall, 1997. A New Class Of Membrane-Bound Chemokine With A CX3C Motif. Nature, 385: 640-4.

2. Bjerkeli, V., J.K. Damås, B. Fevang, J.C. Holter, A. Pål And S.S. Frøland,. 2007. Increased Expression OfFractalkine (CX3CL1) And Its Receptor, CX3CR1, In Wegener's Granulomatosis—Possible Role In VascularInflammation. Rheumatology, Doi:10.1093/Rheumatology/Kem168. Journals.Permissions@Oxfordjournals.Org
3. Bombardier, C., D.D. Gladman, M.B. Urowitz, D. Caron, C.H. Chang And The Committee On PrognosisStudies In SLE, 1992. Derivation Of The SLEDAI: A Disease Activity Index For Lupus Patients. Arthritis Rheum,35: 630-40.
4. Cockwell, P., S.J. Chakravorty, J. Girdlestone And C.O. Savage, 2002. Fractalkine Expression In HumanRenal Inflammation. J. Pathol., 196: 85-90.
5. Dean, G.S., J. Tyrrell-Price, E. Crawley And D.A. Isenberg 2000. Cytokines and Systemic LupusErythematosus. Ann Rheum Dis., 59: 243-51.
6. Hasegawa, M., S. Sato, T. Echigo, Y. Hamaguchi, M. Yasui And K. Takehara, 2005. Up RegulatedExpression Of Fractalkine/CX3CL1 And CX3CR1 In Patients With Systemic Sclerosis. Ann. Rheum. Dis., 64: 21-8
7. Ho, C.Y., C.K. Wong, E.K. Li, L.S. Tam And W.K. Clam, 2003. Elevated Plasma Concentrations Of NitricOxide, Soluble Thrombomodulin And Soluble Vascular Cell Adhesion Molecule-1 In Patients With Systemic LupusErythematosus. Rheumatology, 42: 117-122.
8. Lucas, A.D., N. Chadwick, B.F. Warren, D.P. Jewell, S. Gordon, F. Powrie And D.R. Greaves, 2001. TheTransmembrane Form Of The CX3CL1 Chemokine Fractalkine Is Expressed Predominantly By Epithelial CellsinVivo. Am. J. Pathol., 158: 855-66.
9. Melnicoff, M.J., 1993. Immunofluorescence Methods For Microscopic Analysis. En: Howard Gced.Methods In Non Radioactive Detection. Appleton &Lnge.
10. Pan, Y., C. Lloyd, H. Zhou, S. Dolich, J. Deeds, J.A. Gonzalo, J. Vath, M. Gosselin, J. Ma, B. Dussault,E. Woolf, G. Alperin, J. Culpepper, J.C. Gutierrez-Ramos And D. Gearing, 1997. Neurotactin, A MembraneanchoredChemokine Upregulated In Brain Infl Ammation. Nature, 387: 611-7.
11. Pizarro, S., J.M. R.A. Espino L.J. Jara, R.N. Carlos, 2007. Soluble Vascular Cell Adhesion Molecule-1Indicates SLE Disease Activity And Specific Organ Involvement. Revista Alergia México, 54(6): 189-95.
12. Ruiz-Irastorza, G., M.A. Khamashta, G. Castellino And G.R. Hughes, 2001. Systemic Lupus Erythematosus.Lancet, 357: 1027-32.
13. Sato, E., N. Iikuni, N. Kamatani, H. Okamoto, T. Yoshio And S. Minota, 2006. Soluble Fractalkine In TheCerebrospinal Fluid Of Patients With Neuropsychiatric Lupus. Ann Rheum Dis. September, 65(9): 1257-1259.
14. Tan, E.M., A.S. Cohen, J.F. Fries, *Et Al*., 1982. The Revised Criteria For The Classification Of Systemic LupusErythematosus. Arthritis Rheum, 25: 1271-7.
15. Tillet, W.S. And T.J. Francis, 1930. Serological Reactions In Pneumonia With A Non Portion Somatic FractionOf Pneumococcus. J. Exper. Med., 52: 561-571.
16. Umehara, H., E. Bloom, T. Okazaki, N. Domae And T. Imai, 2001. Fractalkineand Vascular Injury. TrendsImmunol., 22: 602-7.
17. Umehara, H., E.T. Bloom, T. Okazaki, Y. Nagano, O. Yoshie And T. Imai, 2004. Fractalkine In VascularBiology: From Basic Research To Clinical Disease. Arterioscler. Thromb. Vasc. Biol., 24: 34-40.
18. Yajima, N., T. Kasama, T. Isozaki, T. Odai, M. Matsunawa, M. Negishi, H. Ide, Y. Kameoka, S. Hirohata and M. Adachi, 2005. Elevated Levels of Soluble Fractalkine In Active Systemic Lupus Erythematosus: PotentialInvolvement In Neuropsychiatric Manifestations. Arthritis Rheum, 52: 1670-5.
19. Yoshimoto S, Nakatani K, Iwano M, Asai O, Samejima K, Sakan H,Terada M, Harada K, Akai Y, Shiiki H, Nose M, Saito Y. Elevated Levels Of Fractalkine Expression And Accumulation Of CD16_ Monocytes InGlomeruli Of Active Lupus Nephritis. Am J Kidney Dis 50: 47–58, 2007.
20. Tadros Y, Ruiz-Deya G, Crawford BE, Thomas R, Abdel-Mageed AB.In Vivo Proteomic Analysis Of Cytokine Expression In Laser Capturemicrodissected Urothelial Cells Of Obstructed Ureteropelvic Junction Procured By Laparoscopic Dismembered Pyeloplasty. J Endourol 17: 333–336,2003.
21. Wittwer,C.T, Hermann,M.G, Moss,A.A. And Rasmussen,R.P.:Continuous Fluorescence Monitoring Of Rapid Cycle DNA Amplification. Biotechniques,22:130-1,134-8,1997.
22. Ferreira GA, Teixeira AL And Sato EI: Atorvastatin Therapy Reduces Interferon-Regulated Chemokine CXCL9 Plasma Levels In Patients With Systemic Lupus Erythematosus. Lupus (2010) 19, 927–934.
23. Brianjones, Alisae.Koch, And Salahuddinahmed:Pathological Role Of Fractalkine/CX3CL1 In Rhumatic Disease A Unique Chemokine With Multiple Function.Frontier In Immunology.,Vol.2,Article B2, 2012
24. Gemma E. White, David R. Greaves: Fractalkine: A Survivor's Guidearteriosclerosis, Thrombosis, And Vascular Biology. Brief Reviews 2012; 32: 589-594.
25. Wenping Sun, Yulian Jiao, Bin Cui, Xuejun Gao, Yu Xia And Yueran Zhao: Immune Complexes Activate Human Endothelium Involving The Cell-Signaling HMGB1-RAGE Axis In The Pathogenesis Of Lupus Vasculitis. Laboratory Investigation, (29 April 2013) | Doi:10.1038/Labinvest.2013.

9/22/2013