Egy. J. Pure & Appl. Sci. 2018; 56(2):29-35

Egyptian Journal of Pure and Applied Science

Arginase activity in systemic lupus erythematosus patients: A biochemical study

Mohamed Khaled Mohamed[1*] and Amr Aly Elshormilisy[2]

[1]*Ain Shams University Hospitals, Ain Shams University, Cairo, Egypt,*
[2]*Internal Medicine Department, Helwan University, Cairo, Egypt.*

ARTICLE INFO

Article history:
Received 04 October 2018
Accepted 25 December 2018

Keywords:
Systemic lupus erythematosus;
Arginase;
NO;
Atherosclerosis.

ABSTRACT

Recent studies on human and murine models of systemic lupus erythematosus (SLE) have pointed towards the marked role of arginase in the etiology and pathogenesis of this disease. In this study, we measured serum total arginase activity in 40 SLE patients and 32 healthy control subjects. We found a marked elevation in serum arginase activity compared to control subjects. Urea is significantly elevated in patients with serum arginase activity above the median level. A negative correlation was noted between arginase and nitric oxide (NO) in the sera of SLE patients with the latter being significantly lowered compared to control subjects. Serum arginase activity was significantly correlated with markers of disease activity (erythrocyte sedimentation rate, complement component 4 (C4), total leucocyte count and proteinuria) and atherosclerosis (Cholesterol, triacylglycerols, low density lipoproteins and homocysteine). These findings indicate the elevation of serum arginase activity in SLE patients and this elevation is associated with uremia and downregulation of serum NO. Further, our data provide a direct evidence demonstrating a pathogenic role of arginase in SLE since it was highly associated with disease activity and atherosclerosis and suggests that targeting arginase may offer a potential therapeutic strategy for the treatment of SLE.

Introduction

Arginine is a cationic, semi-essential amino acid that is involved in numerous physiological processes. Arginase is an enzyme that hydrolyzes arginine to ornithine and urea. Arginase activity has two major homeostatic purposes: first, to rid the body of ammonia through urea synthesis, and the second, to produce ornithine, the precursor for polyamines and pralines. Polyamines are needed for cell proliferation, collagen formation and other physiologic functions [1]. Increased arginase activity results in increased production of polyamines which stimulates the proliferation of blood vessels smooth muscle cells. Increased proliferation and migratiom of smooth muscle cells are involved in the formation of atherosclerotic plaques [2,3]. Arginine is also the substrate of nitric oxide synthase enzyme to produce citrulline and nitric oxide (NO) [4]. NO plays a critical role in the circulation by decreasing vascular tone, platelet and leukocyte activation, smooth muscle cell proliferation, extracellular matrix deposition, and endothelial cell death.

* Corresponding author.
E-mail address: Khaled61_M@hotmail.com

Decreased bioavailability of NO is a common mechanism involved in the pathogenesis of various vascular disorders, including hypertension, atherosclerosis, diabetes, and ischemia-reperfusion injury [5]. Interestingly, clinical and experimental studies during the past decade demonstrate that arginine administration restores NO synthesis and vascular function in several cardiovascular diseases, suggesting that impaired arginine availability underlies these vascular pathologies [6-8].

In the last years arginase is believed to be a new key player in the human autoimmune diseases. Its up-regulation was observed in patients with SLE, rheumatoid arthritis, autoimmune encephalomyelitis and autoimmune diabetes [9-14]. In SLE patients the autoimmune response is characterized by circulating autoantibodies secreted by B cells, which are aided by IL17-secreting T-cells (Th17). A growing body of evidence in both human and murine studies confirm a pivotal role for Th17 cells in the progression of SLE. Indeed, lupus-prone mice are partially protected from immunopathology by a reduction in renal Th17 cell accumulation.

Very recently, Myeloid-derived suppressor cells (MDSCs) appeared to exacerbate the pathological role of Th17 cells in SLE by an arginase dependent manner. In an in vitro study, the effect of arginase on Th17 was abrogated by the presence of an arginase inhibitor [12,13,15]. Here, we studied serum total arginase activity in a group of patients with SLE in comparison with healthy control subjects. In order to study the pathogenic role of arginase we searched its correlation with urea, serum NO, and biomarkers of disease activity and atherosclerosis.

Subjects and Methods

Study design

Forty patients fulfilling the 1982 American College of Rheumatology criteria [16] for the classification of SLE were recruited in the current study. They were attending the outpatient clinic, Rheumatology unit, Ain Shams University Hospitals. Patients gave informed permission before entering the study. Thirty-two healthy control subjects were also included. Inclusion criteria were as follows: adult SLE patients, single gender (female), and receiving standard treatments. Sera were not hemolyzed to avoid interference of arginase in red blood cells. Exclusion criteria were the presence of other autoimmune diseases and age above 60 years.

Total arginase activity assay

Arginase was determined colorimetrically [17,18] by a kit obtained from Biodiagnostic, Egypt. In this method 10.0 μl of sera were incubated at 37°C for 60 minutes with 10.0 μl of Mn^{2+}, 100 mmol/l, (as an activator) and 200 μl of arginine, substrate, 1.0 mol/l, in carbonate buffer (200 mmol/l) pH 9.5 in glass tubes. Urea standard (50 mg/dl) and reagent blank were treated similar to the test serum. After the previous incubation, 1.0 ml of an acid mixture (sulfuric acid 1.0 mol/l and phosphoric acid 4.0 mol/l) was added to the sera, standard and blank tubes. Next,0.5 ml of diacetyl monoxime (50 mmol/l) was added. The glass tubes, containing the reaction mixture, was incubated for 10 minutes in a boiling water bath. Color developed, which represents arginase activity(U/L), was determined colorimetrically against reagent blank at 525 nm.

Nitric oxide measurement

One means to investigate NO formation is the colorimetric measurement of nitrite (NO_2^-), which is a nonvolatile breakdown product of NO. The method used in this work relies on a diazotization reaction for the detection of NO_2^- that was originally described by Griess in 1879[19]. Griess assay reagents were obtained from Biodiagnostic (Egypt). They were sulfanilamide (10 mmol/l) and N-(1-naphthyl)-ethylenediamine, NED, (1mmo/l) and standard sodium nitrite (50 µmol/l). Sulfanilamide and NED compete for nitrite in the Griess reaction; thus, greater sensitivity is achieved when the two components are added sequentially. Sulfanilamide Solution is added to the sample first, incubate for 5–10 minutes, then NED Solution is added. The resulting azo dye a bright reddish color which is measured at 540 nm. Nitrite level was determined as µmol/l.

Assay of human homocysteine in human sera

A commercially available kit from Bioassay technology Laboratory (China) was used for the measurement of serum homocysteine by enzyme-linked immunosorbent assay (ELISA). Diluted sera were added to homocysteine monoclonal antibodies precoated ELISA wells. After incubation a biotin-conjugated anti-human homocysteine is added. Unbound conjugate is washed away. Strepatvidin HRP is added and binds to biotin conjugated anti-human homocysteine antibody. After incubation unbound streptavidin-HRP is washed away. Substrate solution is then added, and color develops in proportion to the amount of human homocystein. Reaction is ended by adding acid stop solution and absorbance was measured at 450nm. Homocysteine concentration was determined as mmol/ml.

Assay of other biochemical parameters

Total cholesterol (TC), triacylglycerols (TAG) and high-density lipoprotein cholesterol (LDL-c) were measured colorimetrically using commercial assays (Biosystems, Italy). Low density lipoprotein cholesterol was calculated using Friedewald[20] formula, LDL-c = cholesterol – HDL cholesterol – (TAG/5). Kinetic methods were used for the detection of alanine amino transferase (ALT) and aspartate aminotransferase (AST) (Biosystems, Spain). Kinetic colorimetric method (Fixed rate) was used for creatinine assay in serum and urine (Chema Diagnostica, Italy). Urea was determined colorimetrically by a kit obtained from Chema Diagnosyica (Italy). complement components 3 and 4 (C3 and C4) were determined by an immune-turbidity technique (Spimreact, Spain).

Urine total protein was determined by a trichloro-acetic acid turbidimetric method [21]. Trichloro-acetic acid was obtained from Egychem (Egypt). Routine laboratory techniques were used for the detection of erythrocyte sedimentation rate (ESR) and the count of platelets and total leucocytes.

Statistical analysis was performed using the SPSS 11.0 program.

Results and discussion

In the current study, the mean total arginase activity was increased in SLE patients when compared to healthy control subjects (526.07±204.2 *versus* 240.7 ± 34.4, $p<0.00001$) (**Table 1 and Fig. 1**). Arginase activity was not linked to the age or disease duration of our SLE patients (**Table 1**). Three groups of researchers found increased arginase activity in the sera and MDSCs cells of patients with SLE and humanized models of SLE [12,13,22]. However, in another older study by Ruiz [23], arginase activity did not increase in the sera of their group of SLE patients.

Arginase are present almost exclusively in the cytosol of hepatic cells (arginase 1). However, arginase 1expression has been demonstrated in extra-hepatic tissues including endothelial cells and vascular smooth muscle cells. Arginase II is a mitochondrial enzyme with a wide distribution and is expressed in the kidney, prostate, gastrointestinal tract, and the vasculature. Both isoforms

of arginase are present in human sera [24]. Since arginase is a marker of any damage to hepatocytes [25,26], we could exclude, in the current study, the liver as a source of elevated serum arginase activity since there was no inflammation in liver cells (no significant change in the level of liver enzymes of SLE patients compared to normal subjects) (**Table 1**). Moreover, no significant correlation was observed between arginase and liver enzymes. Certainly, further studies are needed to define the source of increased serum arginase activity.

In the next part of this study we measured urea since its elevation is a valuable measure of arginase catabolic effect on arginine [27]. A marked elevation in the mean level of urea was observed in SLE patients compared to normal subjects **(Table 1**). The mean urea concentration in patients was linked to the median of arginase activity (480 *U/l*) since it was significantly elevated in patients with ≥480 *U/l* compared to patients with arginase activity below the median, 62.7 mg/dl, *SD* 16.5 (range 39-95) vs 32.2 *mg/dl*, *SD* 6.8 (range 24-42) & $p<0.00001$, respectively. Moreover, we found a significant correlation between arginase activity and urea concentration (**Table 2**). The significant positive correlation between arginase activity and urea not only confirms the upregulation of arginase in our patients but also highlights on the toxic consequences of elevated urea concentration in those patients. The main toxic consequence of urea elevation is the development of protein carbamylation; a nonenzymatic posttranslational modifications of proteins that is caused by nonenzymatic binding of isocyanate derived from urea dissociation to free amino groups of proteins. This modification has an adverse reaction, since it induces alterations of protein and cell properties. The change in the tertiary structure of proteins can causes the generation of new epitopes and the consequent production of autoantibodies [28-31]. However, it should be noted that protein carbamylation may not be related solely to urea but also to cyanate which is generated by myeloperoxidase and peroxide-catalyzed oxidation of thiocyanate (derived from diet and smoking) at sites of inflammation [32]. Interestingly, anti-carbamylated protein antibodies were detected in SLE patients in a recent study by Ceccarelli *et.al.* [29]. They found significant association between anti-crabamylated protein antibody and erosive damage in SLE-related arthritis (in terms of frequency and severity). They further suggested that these antibodies can represent a biomarker of severity in patients with SLE with joint involvement. Indeed, this observation led us to compare arginase activity with the standard laboratory measures of disease activity. To determine the relation between arginase and disease activity, we studied its correlation with ESR, C3, C4, P/C ratio, peripheral count of platelets and leucocytes (**Table 3**). Of these variables, serum arginase activity correlated positively with ESR and P/C ratio and negatively with white cell count and C4. These findings indicated a significant association between serum arginase activity and the standard laboratory markers of disease activity in SLE patients and confirmed similar findings of other studies in SLE and murine models [12,13,22]. Interestingly, and due to its important role in triggering inflammation in SLE patients, Flynn et al. [33] described arginase in addition to MDSCs and a tumor necrosis factor member, CD95L, as an explosive cocktail in SLE.

Table 1. Characteristics of systemic lupus erythematosus patients and comparison with normal controls

	Healthy controls	**Patients**	***P***
Number	32	40	
Sex *(Female/male)*	32/0	40/0	
Age *(years)*	34.8±8.1(21-51)[1]	31.8±9.1(20-50)	NS[2]
Disease duration *(years)*	---	3.05±1.3 (1-6)	
Arginase *(U/L)*	240.7±34.4 (212 – 297)	526.07±204.2 (261 -995)	0.00001*
Urea *(mg/dl)*	32.25±6.8 (24-42)	49.2±17.1 (28-95)	0.00004*
ALT[3] *(U/l)*	18.4±4.5 (11-28)	19.1±3.7 (12-27)	NS
AST[4] *(U/l)*	20.6±5.3 (11-29)	20.8 ±5.2 (14-29)	NS
Nitric oxide *(µmol/l)*	32.06±7.1 (22-42)	31.7±13.3 (10-60)	NS
Creatinine (*mg/dl)*	0.88 ±0.11 (0.7-1.1)	0.99±0.27 (0.7-1.8)	0.02*
Homocystein *(mmol/ml)*	3.6± (0.7-5.1)	23.1±16.6 (8-66)	0.00001*
Total cholesterol *(mg/dl)*	153±16.7 (140-182)	213±65.6 (111-384)	0.00001*
Triacylglycerols *(mg/dl)*	137±31.1 (90-190)	199±75.2 (100-389)	0.00002*
HDL[5] *(mg/dl)*	66.2±6.9 (55-81)	63.2±6.3 (55-79)	0.03*
LDL[6] *(mg/dl)*	109.6±11.9 (86-135)	141.1±30.3 (100-199)	0.00001*
Protein/creatinine ratio	---	1.4±0.9 (0.01-3.1)	
C3 *(mg/dl)*	110 ±20.3 (84-159)	109±20.8 (80-150)	NS
C4 *(mg/dl)*	32.0± (25-40)	27.2± (11-44)	0.006*
Platelets count[8]	217.5± 43 (150-280)	221±52.6 (150-300)	NS
ESR[7] *(mm/hour)*	21.8± (16-30)	48.8± (19-90)	0.00001*
Total leucocytes count[8]	7.4±1.7 (4.1-10)	5.5±2.1 (3-10.1)	0.00009*

[1]Results are presented as mean ± standard deviation and range. [2] Non-significant. [3]Alanine aminotransferase. [4]Aspartate aminotransferase. [5]High-densitylipoproteins. [6]Low-density lipoproteins. [7]Erythrocyte sedimentation rate. [8]Result x 1000. *A significant result. $P < 0.05$ is considered statistically significant.

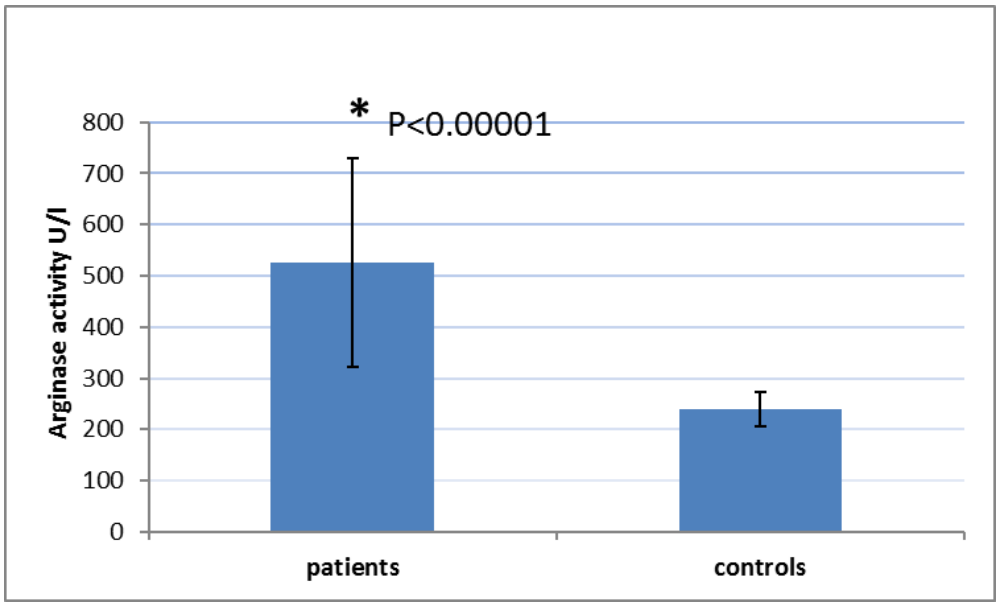

Fig 1: Mean of arginase activity in SLE patients and controls

Table 2. Correlation between arginase activity and urea, nitric oxide, disease duration, age and liver enzymes in SLE patients

	r[1]	***p***
Urea*(mg/dl)*	0.7	0.00001*
Nitric oxide (*µmol/l*)	- 0.71	0.0001
Disease duration	-0.05	NS
Age	0.06	NS
Aspartate aminotransferase (AST)	0.1	NS
Alanine aminotransferase (ALT)	0.09	NS

*significant result. **non-significant result. [1]correlation coefficient**.**
P < 0.05 is considered statistically significant.

Table 3. Correlation between arginase activity and markers of disease activity in SLE patients

	r[1]	***p***
ESR	**0.83**	**0.0001**
Protein/creatinine ratio	**0.48**	**0.001***
C3	**-0.25**	**NS****
C4	**-0.66**	**0.0001**
Platelets count	**-0.057**	**NS**
Total leucocytes count	**-0.52**	**0.0005**

*significant result. **non-significant result. [1]correlation coefficient**.**
P < 0.05 is considered statistically significant.

Consideration of the biochemical properties of arginase and nitic oxide synthase supports the notion that arginase may inhibit the synthesis of NO by competing with NOS for arginine. Although the affinity of arginine is much higher for purified nitic oxide synthase (Km ~ 2–20 µM) than for arginase (Km ~ 1–5 mM), the maximum activity of arginase is about 1,000 times that of nitic oxide synthase [34]. Thus, Sufficient quantities of arginase can limit the availability of arginine for NO synthesis by intact cells. The increased production of urea, which results from upregulation of arginase, is another way by which arginase arginase inhibit the production of NO since urea inhibit nitric oxide synthase by a posttranscriptional mechanism [24]. In the present study, we observed a significant negative correlation between serum NO level and arginase activity in SLE patients which, certainly, reflects the inhibitory effect of arginase on NO production (**Table 2 and Fig. 2**). Interestingly, experimental and *in vitro* researches studied the negative correlation between arginase and NO and found that inhibition of arginase or supplementation of arginine results in augmentation of NO production [34-37].

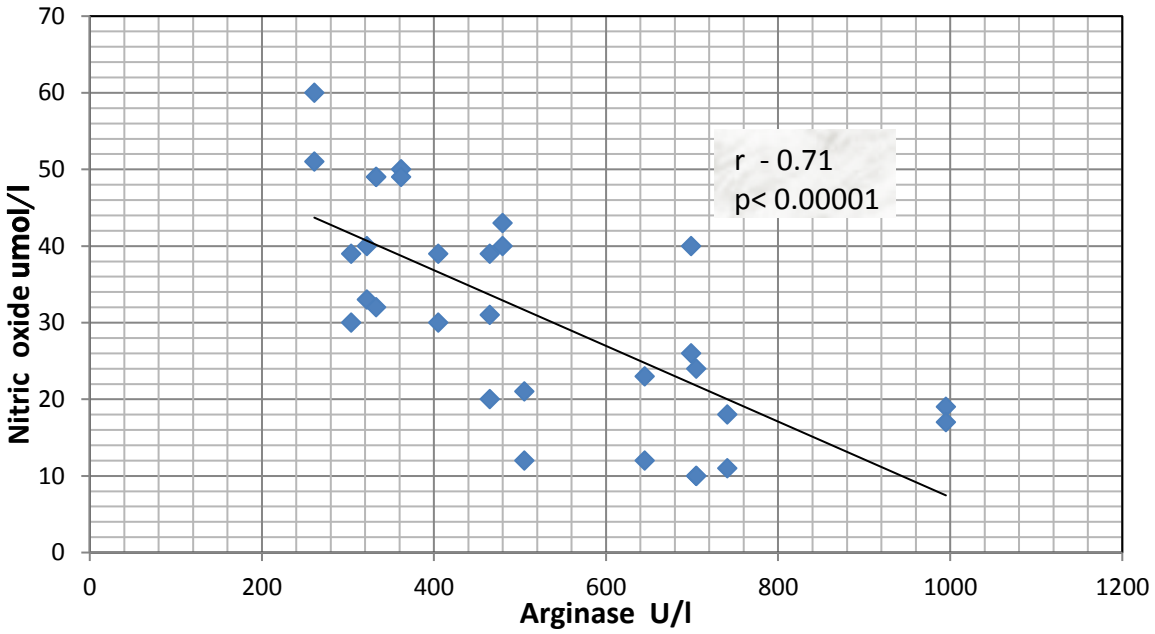

Fig 2: Correlation between arginase activity and nitric oxide in SLE patients

Patients with SLE have a significantly increased risk of cardiovascular morbidity and mortality, particularly related to premature atherosclerosis. Recent discoveries have revealed that endothelial nitric oxide as an important vasoprotective molecule contributing to endothelial functions and that impaired nitric oxide signaling associated with endothelial dysfunction is now regarded as an early marker of atherosclerosis and cardiovascular diseases [38-41]. Given that arginase competes with nitric oxide synthase enzyme for their common substrate, arginine, it is reasonable to assume that increased arginase activity is an important factor in the development of vascular diseases and atherosclerosis [41]. Moreover, Increased arginase activity results in increased production of polyamines which induce proliferation and migration of smooth muscle cells that are involved in the formation of atherosclerotic plaques [2,3].

Consequently, we compared arginase activity with some traditional atherogenic risk factors (total cholesterol, triacylglycerols, HDL and LDL) and homocysteine as a non-traditional risk factor (**Table 4**). Firstly, it must be noted that the mean level of these risk factors was significantly elevated in SLE patients compared to healthy control subjects, with exception of HDL which significantly lowered in patients. Correlation studies revealed a significant positive correlation between serum arginase activity and the traditional risk factors, total cholesterol, triglycerides, and LDL. A significant positive correlation was also found with homocysteine, as a non-traditional risk factor. Though they did not measure arginase, the study of Gujjarlamudi*et.al.* [42], to a great extend supported our results. They found that arginine supplementation for 15 days to a group of 25 SLE patients has restored their vascular function and improved the clinical symptoms in patients with vascular dysfunction and atherosclerosis. They attributed this improvement to increasing the bioavailability of NO.

In conclusion, the results presented here showed a marked increase in serum arginase activity in a group of 40 SLE patients. A marked elevation in serum urea was observed, especially in patients with arginase activity above the median level. Our study further defined the role of arginase in the pathogenesis of SLE through its inverse correlation with NO and positive correlation with markers of disease activity and atherosclerosis.

Table 4. Correlation between arginase activity and markers of atherosclerosis SLE patients

	R[1]	**p**
Homocystein	0.58	0.0008*
Cholesterol	0.47	0.002
Triacylglycerols	0.40	0.001
High-density lipoproteins	-0.1	NS**
Low-density lipoproteins	0.7	0.00001

*significant result. **non-significant result. [1]correlation coefficient.
P < 0.05 is considered statistically significant.

Recommendations

1) Further studies on a larger number of SLE patients are recommended to evaluate the exact prevalence of increased arginase activity in addition to defining its triggering factor(s) and the extrahepatic origin.
2) The assay of serum urea and NO should be a part of the routine investigations performed for SLE patients during follow-up.
3) Carbamylation and arginase, a lot of researches is needed to clearly define their correlation.
4) Arginase could be a new target for future therapy for SLE, thus, extensive trials are needed to this effect.

References

1) **Caldwell, R. B., Toqued, H. A., Narayanan, S. P. and Caldwell, R. W. (2015).** Arginase: an old enzyme with new tricks. Trends Pharmacol Sci. **36**(6):395–405.
2) **Rabelo, L. A., Ferreira, F. O., Nunes-Souza, V., da Fonseca, L. J. and Goulart, M. O. F. (2015).** Arginase as a Critical Prooxidant Mediator in the Binomial Endothelial Dysfunction-Atherosclerosis. Oxidative Medicine and Cellular Longevity. http://dx.doi.org/10.1155/2015/924860.
3) **Xiong, Y., Yu, Y., Montani, J., Yang, Z. and Ming, X. (2013).** Arginase-II Induces Vascular Smooth Muscle Cell Senescence and Apoptosis Through p66Shc and p53 Independently of Its l-Arginine Ureahydrolase Activity: Implications for Atherosclerotic Plaque Vulnerability. J Am Heart Assoc. **2**: e000096 doi: 10.1161/JAHA.113.
4) **Rath, M., Müller, I., Kropf, P. and Ellen. (2014).** Metabolism via arginase or nitric oxide synthase: two competing arginine pathways in macrophages Closs and Markus Munder. Frontiers in immunology. **27:** 1-10.
5) **Pacher, P., Beckman, J. S. and Liaudet, L. (2007).** Nitric oxide and peroxynitrite in health and disease. Physiol Rev **87**: 315–424.
6) **John, S. and Schmeider, R. E. (2003).** Potential mechanisms of impaired endothelial function in arterial hypertension and hypercholesterolemia. Curr Hypertens Rep. **5**:199–207.
7) **Soriano, F. G., Virag, L. and Szabo, C. (2001).** Diabetic endothelial dysfunction: role of reactive oxygen and nitrogenspecies production and poly (ADP-ribose) polymerase activation. J Mol Med. **79**:437–448.
8) **Kawashima, S. and Yokoyama, M. (2004).** Dysfunction of Endothelial Nitric Oxide Synthase and Atherosclerosis Arterioscler Thromb Vasc Biol. **24**: 998-1005.
9) **Hernandez, L. F., Buchwald, P. and Abdulreda, M. H. (2018).** Effect of Arginase-1 Inhibition on the Incidence of Autoimmune Diabetes in NOD Mice. Curr Res Diabetes Obes J., **5**(3): 1-13.
10) **Hernandez, L. F., Buchwald, P. and Abdulreda, M. H. (2018).** Effect of Arginase-1 Inhibition on the Incidence of Autoimmune Diabetes in NOD Mice. Curr Res Diabetes Obes J. **5**(3):1-13.
11) **Xu, L., Hilliard, B., Carmody, R. J., Tsabary, G., Shin, H., Christianson, D. W. and Chen, Y. H. (2003)**. Arginase and autoimmune inflammation in the central nervous system. Immunology, **110**(1):141-148.
12) **Zhang, D., Xu, J., Ren, J., Din, L., Shi, G., Li, D., Dou, H. and Hou, Y. (2018).** Myeloid-Derived suppressor cells induce Podocyte injury Through increasing reactive Oxygen species in lupus nephritis. Frontiers in immunology, **9**: 1-12.
13) **Wu, H., Zhen, Y., Ma, Z., Li, H., Yu, J., Xu, Z., Wang, X., Yi, H. and Yang, Y. (2016).** Arginase-1–dependent promotion of TH17 differentiation and disease progression by MDSCs in systemic lupus erythematosus. Sci Transl Med. **8**: 1-23.
14) **Jiao, Z., Hua, S., Wang, W., Wang, H., Gao, J. and Wang, X. (2013).** Increased circulating myeloid-derived suppressor cells correlated negatively with Th17 cells in patients with rheumatoid arthritis. Scand J Rheumatol. **42**:85-90.
15) **Wang, D., Huang, S., Yuan, X., Liang, J., Xu, R., Yao, G., Feng, X. and Sun, L. (2017).** The regulation of the Tre/Th17 balance by mesenchymal stem cells in human systemic lupus erythematosus. Cell Mol Immunol. **14**: 423–431.
16) **Tan, E. M., Cohen, A. S., Fries, J. F., Masi, A. T., McShane, D. J., Rothfield, N. F., Schaller, J. G., Talal, N. and Winchester, R. J. (1982).** The 1982 revised criteria for the classification of systemic lupus erythematosus. Arthritis Rheum. **25**:1271–1277.
17) **Marsh, W. H., Fingerhut, B. and Miller, H. (1965).** Automated and Manual Direct Methods for the Determination of Blood Urea. Clinical Chemistry. **11**: 624-627.
18) **Jergović, I., Zuzić, I., Fiser-Herman, M. and Straus, B. (1970).** A simple method for serum arginase determination. Clin Chim Acta. **30**: 765-74.
19) **Griess, P. (1879)**. Bemerkungenzu der abhandlung der H.H. Weselsky und Benedikt "Uebereinigeazover bindungen." *Chem. Ber.* **12,** 426–428.
20) **Friedewald, W. T., Levy, R. I. and Fredrickson, D. S. (1972).** Estimation of the concentration of low-density lipoprotein cholesterol in plasma, without use of the preparative ultracentrifuge. Clinical Chemistry, **18**: 499–502.
21) **Henry, R. J., Sobel, C. and Segalove, M. (1956).** Turbidimetric determination of proteins with sulfosalicylic and trichloracetic acids. Proc SOC Exp Biol Med. **92**:748-751.
22) **HUANFA, Y. I., Wu, H., Zhen, Y., Ma, Z., Li, H. and Yang, Y. (2016).** MDSCs enhance Th17 differentiation and disease progression through arginase-1 production in patients with systemic lupus erythematosus. J Immunol., **196**: 124-132.
23) **Ruiz, B. E. (2009).** Seric arginase in patients with systemic lupus erithematosus. Rev Mex Patol Clin. **56**:45-49.

24) **Durante, W., Johnson, F. K. and Johnson, R. A. (2007).** Arginase: A critical regulator of nitric oxide synthesis and vascular function. Clin Exp Pharmacol Physiol. **34**: 906–911.
25) **Ikemoto, M., Ishida,' A., Tsunekawa, S., Ozawa, K., Kasai, Y., Totani, M. and Ueda, K. (1993).** Enzyme Immunoassay of Liver-Type Arginase and Its Potential Clinical Application. Clin. Chem. **39**: 794-799.
26) **Ikemoto, M., Tsunekawa, S., Toda, Y. and Totani, M. (2001).** Liver-Type Arginase Is a Highly Sensitive Marker for Hepatocellular Damage in Rats, Clinical Chemistry **47**: 946-948.
27) **Morris, S. M. Jr. (2009).** Recent advances in arginine metabolism: roles and regulation of the arginases. British Journal of Pharmacology **157,** 922–930.
28) **Gorissea, L., Pietrementa, C., Vuibleta, V., Schmelzer, C. E. H., Köhler, M., Duca, L., Debellea, L., Fornèsg, P., Jaissona, S. and Gillerya, P. (2016).** Protein carbamylation is a hallmark of aging. PNAS. **113**: 1191–1196.
29) **Ceccarelli, F., Perricone, C., Colasanti, T., Massaro, L., Cipriano, E., Pendolino, M., Natalucci, F., Mancini, R., Spinelli, F. R. Valesini, G., Conti, F. and Alessandri, C. (2018).** Anti-carbamylated protein antibodies as a new biomarker of erosive joint damage in systemic lupus erythematosus. Arthritis Research & Therapy **20**:126-136.
30) **Drechsler, C., Kalim, S., Wenger, J. B., Suntharalingam, P., Hod, T., Thadhani, R. I., Karumanchi, S. A., Wanner, C. and Berg, A. H. (2015).** Protein carbamylation is associated with heart failure and mortality in diabetic patients with end-stage renal disease.Kidney Int. **87**:1201-1208.
31) **Badar, A., Arif, Z. Alam, K. (2018).** Role of Carbamylated Biomolecules in Human Diseases. IUBMB Life. **70**: 267-275.
32) **Kalim, S., Karumanchi, S. A., Thadhani, R. I., and Berg, A. H. (2014).** Protein carbamylation in kidney disease: pathogenesis and clinical implications. Am J Kidney Dis., **64**:793–803.
33) **Flynn, R. J., Legembre, P., Gorissea, L. and Pietrementa, C. (2016).** Myeloid-derived suppressor cell, arginase-1, IL-17 and cl-CD95L: an explosive cocktail in lupus? *Ann Transl Med* **4**:554-259.
34) **Guoyao, W. U. and MORRIS, S. M. JR. (1998).** Arginine metabolism: nitric oxide and beyond. Biochem J. **336**: 1-17.
35) **Chang, C. I., Liao, J. C. and Kuo, L. (1998).** Arginase modulates nitric oxide production in activated macrophages. Am J Physiol Heart Circ Physiol **274**: H342–H348.
36) **Hey, C., Boucher, J. L., Vadon-Le G. S, Ketterer, G., Wessler, I. and Racke, K. (1997).** Inhibition of arginase in rat and rabbit alveolar macrophages by N -hydroxy-D,L-indospicine, effects of L-arginine utilization by nitric oxide synthase. Br J Pharmacol. **121**:395–400.
37) **Tenu, J. P., Lepoivre, M., Moali, C., Brollo, M., Mansuy, D. and Boucher, J. L. (1999).** Effects of the new arginase inhibitor N -hydroxy-nor-L-arginine on NO synthase activity in murine macrophages. Nitric Oxide. **3**: 427–438.
38) **Chen, J. Y., Ye, Z. X., Wang, X. F., Chang, J., Yang, M. W., Zhong, H. H., Hong, F. F. and Yang, S. L. (2018).** Nitric oxide bioavailability dysfunction involves in atherosclerosis. Biomed Pharmaco ther. **97**: 423-428.
39) **Tousoulis, D., Kampoli, A. M., Tentolouris, C., Papageorgiou, N. and Stefanadis, C. (2012).** The role of nitric oxide on endothelial function. Curr. Vasc. Pharmacol. **10**: 4-18.
40) **Bauer, V. and Sotníková, R. (2010).** Nitric oxide-the endothelium-derived relaxing factor and its role in endothelial functions.Gen Physiol Biophys. **29**: 319-40.
41) **Nguyen, M. C., Park, J. T., Jeon, Y. G., Jeon, B. H., Hoe, K. L., Kim, Y. M., Lim, H. K. and Ryoo, S. (2016).** Arginase Inhibition Restores Peroxynitrite-Induced Endothelial Dysfunction via L-Arginine-Dependent Endothelial Nitric Oxide Synthase Phosphorylation. Yonsei Med J. **57**: 1329-1338.
42) **Gujjarlamudi, H. B., Rajasekhar, L. and Naidu, M. U. R. (2013).** L-arginine improves vascular function in patients with systemic lupus Erythematosus. International Journal of nutrition, Pharmacology, Neurological Diseases. **3:** 131-134.