Purines and oxypurines in myocardial ischemia

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ABSTRACT

الأهداف: تقييم مستويات نيوكليوسيدات البيورين والاوكسي بيورين كمؤشرات إضافية لتشخيص مرض القلب الإقفاري وشدّة إحتشاء العضلة القلبية.

الطريقة: أجريت دراسة شملت 101 مريضاً، 19 مريض مصاب بالذبحة الصدرية المستقرة، الذبحة الصدرية الغير المستقرة 29 مريض، إحتشاء عضلة القلب 53 مريضاً تم تنويمهم في وحدة أمراض القلب – مستشفى الكاظمية التعليمي – بغداد – العراق، خلال الفترة مابين يناير 2007م وحتى نوفمبر 2007م. إضافة إلى 31 شخص من الأصحاء. تم أخذ عينات دم من المرضى المصابين بإحتشاء عضلة القلب خلال الـ12 ساعة الأولى من تعرضهم لآلام في الصدر. قيست مستويات الأدينوسين (ADO)، الإينوسين (INO)، والهايبوزانثين (HYP)، والزانثين (XAN) بطريقة الكروماتوغراف السائل الفائق الكفاءة في بلازما دم.

النتائج: لوحظ صعود إحصائي بيّن في مستويات كافة (ADO, INO, HYP, XAN) عند مرضى إحتشاء العضلة القلبية الحاد والذبحة الصدرية الغير المستقرة (AMI) مقارنة مع قرائن هذه القيم في كل من مجموعتي الأصحاء والذبحة الصدرية المستقرة. كانت الزيادة معنوية في مستوى INO - p=0.01 و HYP - p=0.001 عند مرضى السكري ولدى الذكور المصابين بإحتشاء العضلة القلبية الحاد وبأعمار تزيد عن 54 عام. سجلت معدلات حامض البوليك زيادة معنوية لدى ذوي ضغط الدم المرتفع الذين يعانون من الذبحة الصدرية الغير المستقرة والمدخنين المصابين بالذبحة الصدرية المستقرة.

خاتمة: إن المستويات المرتفعة لهذه البيورينات ونواتج انتقاضها يعكس هبوط تمثيل الطاقة في مرضى القلب، ومن الممكن استخدامها كمؤشر إضافي مستبق للقصور الدموي أو حادث معه. نقترح ابتداء المعالجة بالنيوكليوسيدات اومشتقات الأوكسي بيورينات لتحسن الأداء الوظيفي للبطين عقب انسداد الشريان التاجي.

Objectives: To evaluate the plasma levels of purine nucleosides and oxypurines in the presence of other risk factors as additional markers for the diagnosis of myocardial ischemia and severity of myocardial infarction.

Methods: A case control study was conducted on 101 patients with ischemic heart disease (stable angina, n=19: unstable angina, n=29: acute myocardial infarction [AMI]; n=53 patients) admitted to the Cardiology Unit at Al-Kadhimyia Teaching Hospital, Baghdad, Iraq from January to November 2007 in addition to 31 healthy controls. Blood samples were aspirated from those with AMI within the first 12 hours of onset of chest pain. Plasma adenosine (ADO), inosine (INO), hypoxanthine (HYP), and xanthine (XAN) were analyzed by high-performance liquid chromatography.

Results: The mean plasma ADO, INO, HYP, and XAN levels were raised in unstable angina over the control values. More increase in all nucleosides and oxypurines was reported in the plasma of patients with AMI as compared to the controls and those of stable angina. The INO (p=0.01) and HYP (p=0.001) values were increased significantly in diabetic men with AMI and at age of ≤54 years. The mean uric acid values were significantly elevated in hypertensives with unstable angina and smokers with stable angina.

Conclusion: The levels of purines and their catabolites could be used as additional indices for prior or current ischemia. Pretreatment with such nucleosides, or their oxypurine derivatives, is suggested to improve the regional ventricular function after coronary artery occlusion.

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Myocardial ischemia is a condition that exists when the fractional uptake of oxygen in the heart is not sufficient to maintain the rate of cellular oxidation, which results in structural and functional abnormalities of the heart as a consequence of an inadequate supply of blood to its tissue.[1] This oxidative stress may damage cellular proteins and cause myocyte apoptosis and necrosis. It is associated with arrhythmias and endothelial dysfunction.[2] Chronic ischemia occurs gradually and silently, and in months or years the ischemic tissue and the rest of the body may undergo biochemical and molecular adaptive modifications to accommodate chronic low flow myocardial hibernation and pre-conditioning.[3] Lu et al[4] observed that lactate severely reduces the contractility of cardiomyocytes in a concentration dependent manner that contributes to the ischemic injury. The amount of the purine degradation products released during ischemia and reperfusion correlates with the infarct size and is inversely proportional to the area of viable myocardium. Zmudka et al,[5] supposed that endogenously released adenosine (ADO) during reperfusion may have a protective effect on myocardium. Adenosine, a potent vasodilator, is also taken into account among many agents that attenuate the myocardial reperfusion injury by improving the myocardial salvage and reducing the infarct size in acute myocardial infarction (AMI) patients and preserve the endothelial function.[6] During ischemia, inosine (INO) is a positive inotropic agent that dilates coronary blood vessels. An INO infusion increases blood flow, resulting in decreased myocardial damage. It is protective against adenosine triphosphate (ATP) loss during ischemia and improves the functional recovery on reperfusion. Inosine may influence carbohydrate uptake, the activity of glycolytic enzymes, and releasing of insulin.[7] Serum hypo-xanthine (HYP) and uric acid (UA) values are increased during episodes of pain in patients with angina (**Figure 1**), which probably reflects the ATP degradation.[8] In the atherosclerotic prooxidative environmental milieu the original antioxidant properties of UA paradoxically become prooxidant, thus contributing to the oxidation of lipoproteins within the atherosclerotic plaques.[9] Uric acid's contribution to atherosclerotic vascular disease, however, is still somewhat controversial. Various mechanisms have been suggested through which UA may be implicated in the atherosclerotic process and its clinical complications. It can act as a prooxidant, particularly at increased concentrations, and may thus be a marker of oxidative stress.[10] So, in this study we measured the plasma nucleosides (ADO and INO) and oxypurine (HYP, XAN, and UA) levels and we explore the association of these markers with the degree of ischemia and location of wall infarction in the presence of other risk factors such as the age, diabetes mellitus, hypertension, and smoking, and to test their use as additional markers in the diagnosis of the jeopardized myocardium.

Methods. The study was conducted on 101 patients (aged 30-80 years) with ischemic heart disease (IHD) attended the cardiology unit at Al-Kadhimyia Teaching Hospital, Baghdad, Iraq during the period from January to November 2007. Among these patients (28 women and 73 men), 53 presented with AMI, 29 patients with unstable angina and 19 patients had classical angina pectoris. The diagnosis of IHD was based on the clinical history of the patients, history of chest pain, ECG changes, and cardiac enzymes alteration.[11] According to the ECG changes (which were reviewed by the senior consultant in cardiology), the AMI patients were grouped into anterior (n=19), inferior (n=22), and extensive anterolateral wall infarction (n=12). The present study encompasses also 31 nonsmoker non-diabetic healthy volunteers aged 27-64 years (12 women and 19 men). Patients with signs of renal impairment (high serum urea and creatinine levels) were excluded from the study. The research protocol was approved by the Ethical Committee at the College of Medicine, Alnahrain University, Baghdad, Iraq. Each patient and control subject was informed of the research objectives and methodology, and an oral personal consent was obtained. Ten to 12 milliliters (mls) of overnight fasting blood were aspirated into EDTA containing test tubes within the first 12 hours of the attack of chest pain in

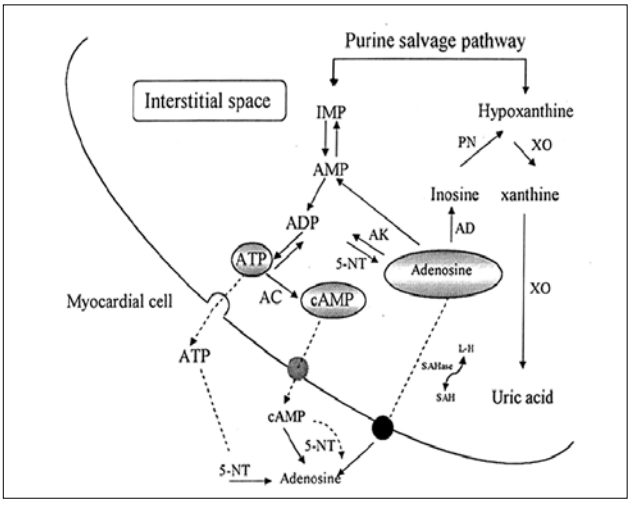

Figure 1 - Purine salvage pathway showing the adenosine triphosphate (ATP) degradation and the release of adenosine hypoxanthine, xanthine, and uric acid IMP - inosine monophosphate, AK - adenylate kinase, AD - adenosine deaminase, XO - xanthine oxidase, AC - adenylate cyclase, PN - polynucleotidase, 5-NT - 5-nucleotidase, SAHase - S-adenosyl homocysteinase, SAH - s-adenosyl homocysteine, LH - l-homocysteine

patients with unstable angina, and AMI. Uric acid, glucose levels, serum cardiac enzymes (total creatinine kinase [CK] and creatinine kinase-myocardial bound [CK-MB] isoenzyme) activities were analyzed by standard laboratory methods.[12] Purine nucleosides (ADO, INO) and oxypurines (HYP, XAN) were separated by high-performance liquid chromatography (HPLC) supplemented with UV-spectrophotometric detector (at 260 nm) and CR-4A chromatopack recorder with computer function. The elution from the M Bondapak column C18 (4.6 x 25 mm, i.d) was carried out by potassium phosphate buffer (10 mM/L, PH=5) at a flow rate of 0.5 ml/min at 45°C.[13]

All statistical analysis was carried out using SPSS version 6. The data were expressed as means±SEM and were analyzed by analysis of variance, student t-test, and linear regression analysis. Differences were considered significant at values of $p<0.05$.

Results. Table 1 depicts some of the clinical and biochemical data of controls and patients with IHD. The mean serum cardiac enzymes (total CK and the

Table 1 - Clinical and biochemical data of controls and patients with ischemic heart disease (IHD).[a]

Clinical and biochemical data	Control (n=31)	Stable angina (n=19)	Unstable angina (n=29)	Acute myocardial infarction (n=53)	AMI subtypes		
					Inferior (n=22)	Anterior (n=19)	Extensive (n=12)
Gender							
Women	12	9	9	10	11	9	5
Men	19	10	20	43	11	10	7
Age (years) (mean±SD)	50 ± 9.52	59 ± 6.7	56 ± 6.5	58 ± 9.5	58 ± 6.0	58 ± 3.0	75 ± 7.0
Diagnostic blood pressure (mm Hg/hour)	76.8 ± 7.0	82.7 ± 9.4	80.2 ± 8.4	81. ± 1 9.7	80 ± 9.1	81.1 ± 7.0	81.4 ± 5.2
Smokers	-	9	15	25	9	8	8
Hypertension	-	13	13	20	5	9	6
Diabetes mellitus	-	11	20	23	9	12	9
Previous history of IHD	-	2	5	6	1	3	2
Time of onset of chest pain (hours)	-	-	14 ± 8.0	12 ± 8.0	12 ± 8.0	12 ± 8.0	12 ± 8.0
Serum glucose (mmol/L)	5.0 ± 0.72	7.9† ± 0.59	7.7† ± 0.46	7.2† ± 0.40	7.2† ± 0.57	6.1† ± 0.46	8.2† ± 1.25
Hb A1c (%)	3.94 ± 0.1	5.9† ± 0.41	5.3†± 0.34	5.4†± 0.25	5.0†± 0.47	5.4†± 0.41	5.8† ± 0.33
Serum urea (mmol/L)	4	4	3.7	5.8	5.1	5.8	6.4
Serum creatinine (µmol/L)	70	65	70	78	76	75	82
Serum total CPK activity(U/L)	145.9 ± 9.9	156.3 ± 12	164.6 ±12.19	568.3 ± 39.3†	485.95†,b*	676.79†	547.33†
Serum CK-MB activity(U/L)	2.5 ± 0.26	2.8 ± .34	3.1± 0.36	27.5 ± 1.10	23.95†	31.87†	27.13†

CPK - creatine phosphokinase, CK-MB - creatinine kinase-myocardial bound, [a]results expressed as mean ± SEM, analysis of variance (ANOVA) between control and IHD groups; †$p<0.001$, [b]ANOVA between inferior and anterior wall infarction; *$p<0.05$. AMI - acute myocardial infarction

Table 2 - The mean ± SEM plasma values of uric acid, adenosine, inosine, hypoxanthine, and xanthine, in controls and patients with stable angina (S-angina), unstable angina (US-angina), acute myocardial infarction (AMI), and different AMI subtypes.[§]

Data	Uric acid	Adenosine	Inosine	Hypoxanthine	Xanthine
Control (n=31)	267.8 ± 17.31	2.38 ± 0.080	0.41± 0.01	0.84±0.030	0.39± 0.015
S-angina (n=19)	333.7*[a] ± 22	2.41 ± 0.13	0.44 ± 0.018	0.87 ± 0.058	0.45 ±0.020
US-angina (n=29)	386.8‡[a] ± 27.4	2.68*[a] ± 0.07	0.50‡[a,†,b] ± 0.015	1.10‡[a,†,b] ± 0.038	0.51‡[a] ±0.019
AMI (n=53)	392.7‡[a,*d] ± 11.31	3.14‡[a,c,d] ± 0.065	0.52‡[a,d] ± 0.008	1.30‡[a,c,d] ± 0.032	0.61‡[a,c,d] ± 0.021
MIS					
Inferior (n=22)	387.8 ± 20.1	3.32 ±0.092	0.53 ± 0.013	1.27 ± 0.054	0.61 ± 0.020
Anterior (n=19)	419.5 ± 14.3	3.15 ± 0.096	0.52 ± 0.015	1.36 ± 0.052	0.62 ± 0.038
Extensive (n=12)	368.4± 23.8	2.95 ± 0.017	0.51 ± 0.016	1.27 ±0.070	0.61± 0.038

MIS - myocardial infarction subtypes, [§]results are expressed in µM/L. [a]ANOVA tests between IHD - sub groups and controls: *$p<0.05$, †$p<0.01$, ‡$p<0.001$. [b]ANOVA tests between US-angina and S-angina groups: *$p<0.05$, ‡$p<0.001$. [c]ANOVA tests between US-angina and AMI groups: *$p<0.05$, ‡$p<0.001$. [d]ANOVA tests between S-angina and AMI groups: *$p<0.05$,†$p<0.01$,‡$p<0.001$.

isoenzyme CK-MB) measured in the first 12 hours of the onset of chest pain in patients with AMI were significantly increased (p<0.001) as compared to those of healthy controls, stable, and unstable angina. Between AMI subgroups, ANOVA test showed that the mean total CK activity was only significantly higher in anterior wall infarction as compared to the inferior wall infarction values (p<0.05), with an obvious but insignificant elevation in the CK-MB isoenzyme activity. **Table 2** reveals the mean ± SEM values of plasma UA, ADO, INO, HYP, and XAN in plasma of controls and patients with IHD and different AMI subtypes. A significant increase in the mean UA was observed in patients with stable angina (p<0.05) with a more significant rise in those with unstable angina and AMI (p<0.001). The levels of nucleosides and oxypurines in patients with stable angina were comparable to the reference values. However, in the unstable angina group, the mean plasma level of ADO was found to increase over that found in the control group (p<0.05). There was a marked and significant elevation in the mean levels of INO, HYP, and XAN (p<0.001) in patients with unstable angina. Significant elevation in the mean values of all these 3 oxypurines (INO, HYP,

Table 3 - Effect of gender, age, diabetes mellitus, hypertension, and smoking on the mean ±SEM plasma adenosine values (μM/L) in controls and patients with ischemic heart disease (IHD).

Parameters	Control	Stable angina	Unstable angina	AMI
Gender				
Women	2.50 ± 0.094	2.49 ± 0.17	2.58 ± 0.13	3.18^{‡,a} ± 0.154
Men	2.26 ± 0.108	2.33 ± 0.20	2.77^{‡,a} ± 0.08	3.10‡ ± 0.072
Age (years)				
≤54	2.51 ± 0.094	2.31 ± 0.17	2.80^{*,a} ± 0.058	3.15^{‡,a} ± 0.098
>54	2.24 ± 0.132	2.51 ± 0.20	2.56,^{*,a} ± 0.11	3.13^{‡,a} ± 0.088
Diabetes mellitus				
Diabetics	-	2.70^{*,b} ± 0.16	2.72 ± 0.08	3.18^{*,d,c} ± 0.094
Non-diabetics	-	2.11 ± 0.13	2.63^{*,c} ± 0.14	3.1^{*,d,c} ± 0.09
Hypertension				
Hypertensive	-	2.50 ± 0.25	2.78^{*,c} ± 0.078	3.16^{*,d,c} ± 0.11
Non-hypertensive	-	2.31 ± 0.14	2.59 ± 0.11	3.12^{*,d,c} ± 0.082
Smoking				
Smokers	-	2.31 ± 0.14	2.59 ± 0.11	3.12^{*,d,c} ± 0.082
Non-smokers	-	2.31 ± 0.14	2.59 ± 0.11	3.12^{*,d} ± 0.082

[a]analysis of variance (ANOVA) tests for gender and age differences between controls and IHD: *p<0.05, $^{\dagger}p$<0.01, $^{\ddagger}p$<0.001.
[b]within group paired t- test: diabetics, hypertensives, smokers versus their respective counter-groups : *p<0.05.
[c]ANOVA tests between US-angina and S-angina : *p<0.05. [d]ANOVA tests between US-angina and AMI: *p<0.05.
[e]ANOVA tests between S-angina and AMI : *p<0.05. AMI - acute myocardial infarction

Table 4 - Effect of gender, age, diabetes mellitus, hypertension, and smoking on the mean (±SEM) plasma inosine values (μM/L) in controls and patients with ischemic heart disease (IHD).

Parameters	Control	Stable angina	Unstable angina	AMI
Gender				
Women	0.42 ± 0.013	0.46 ± 0.082	0.50^{†,a} ± 0.026	0.51^{†,a} ± 0.014
Men	0.40 ± 0.014	0.42 ± 0.038	0.50^{‡,a} ± 0.02	0.52^{‡,a} ± 0.01
Age (years)				
≤54years	0.42 ± 0.014	0.43 ± 0.026	0.50^{†,a} ± 0.024	0.53^{‡,a} ± 0.014
>54 years	0.40 ± 0.012	0.45 ± 0.028	0.50^{†,a} ± 0.02	0.51^{†,a} ± 0.01
Diabetes mellitus				
Diabetics	-	0.43 ± 0.03	0.48^{*,c} ± 0.016	0.54^{*b,†,d} ± 0.01
Non diabetics	-	0.45 ± 0.019	0.52^{*,c} ± 0.034	0.50 ^{*d} ± 0.01
Hypertension				
Hypertensives	-	0.43 ± 0.019	0.52^{†,c} ± 0.024	0.52^{†,d} ± 0.014
Non-hypertensives	-	0.45 ± 0.048	0.48 ± 0.018	0.52^{*d} ± 0.01
Smoking				
Smokers	-	0.42 ± 0.024	0.50^{*,c} ± 0.024	0.51^{*d} ± 0.040
Non smokers	-	0.46 ± 0.030	0.50 ± 0.018	0.53^{*d} ± 0.01

Within group t-test for both gender and age: not significant. [a]analysis of variance (ANOVA) tests for gender and age differences between controls and IHD: *p<0.05, $^{\dagger}p$<0.01, $^{\ddagger}p$<0.001. [b]within group t- test: diabetics, hypertensives, smokers versus their respective counter-groups: *p<0.05. [c]ANOVA tests between US-angina and S-angina: *p<0.05, $^{\dagger}p$<0.01.
[d] ANOVA tests between S-angina and AMI: *p<0.05, $^{\dagger}p$<0.01. AMI - acute myocardial infarction

XAN) was observed in the plasma of patients with AMI as compared to values of the controls and patients with stable angina (p<0.001). Between groups, the ANOVA test revealed significant rise in the mean values of INO and HYP (p<0.01) in those with unstable angina when compared to the corresponding values of the stable angina group. The mean levels of UA, ADO, INO, HYP, and XAN showed insignificant elevation in patients with extensive wall infarction as compared to the mean values of patients with anterior wall or inferior wall myocardial infarction. **Table 3** reveals the effect of gender, age, hyperglycemia (diabetes mellitus), hypertension, and smoking on the means of the plasma ADO levels in the controls and patients with IHD. The mean plasma ADO level in women with stable and unstable angina was comparable to those of women controls, but was significantly elevated in women with AMI (p<0.001). The table also shows that there was no significant gender or age differences in the ADO levels in patients with stable angina or in the normal controls. The mean plasma ADO levels showed a significant elevation in men with AMI and unstable angina (p<0.001) as

Table 5 - Effect of gender, age, diabetes mellitus, hypertension, and smoking on the mean ±SEM plasma hypoxanthine values (μM/L) in controls and patients with ischemic heart disease (IHD).

Parameters	Control	Stable angina	Unstable angina	AMI
Gender				
Women	0.83 ± 0.05	0.82 ± 0.09	1.00*[a] ± 0.07	1.3‡[a] ± 0.092
Men	0.84 ± 0.038	0.91 ± 0.08	1.15‡[a] ± 0.04	1.29‡[a] ± 0.034
Age (years)				
≤54	0.82 ± 0.04	0.87 ± 0.096	1.11†[a] ± 0.064	1.35‡[a] ± 0.05
>54	0.85 ± 0.043	0.87 ± 0.072	1.10*[a] ± 0.044	1.26‡[a] ± 0.044
Diabetes mellitus				
Diabetics	-	0.89 ± 0.078	1.12*[b] ± 0.046	1.26*[c,*d] ± 0.050
Non diabetics	-	0.84 ± 0.092	1.03 ± 0.054	1.33 *[c,*d] ± 0.044
Hypertension				
Hypertensives	-	0.85 ± 0.06	1.11†[b] ± 0.038	1.28*[c,*d] ± 0. 16
Non-hypertensives	-	0.90 ± 0.034	1.10 ± 0.06	1.31*[c,*d] ± 0.042
Smoking				
Smokers	-	0.91 ± 0.060	1.13*[b] ± 0.038	1.28*[d] ± 0. 16
Non smokers	-	0.82 ± 0.014	1.10*[b]± 0.06	1.31*[c,*d] ± 0.042

Within group t-test for both gender and age: not significant. within group t- test: diabetics, hypertensives, smokers versus their respective counter-groups: no significant differences. [a]analysis of variance (ANOVA) tests for gender and age differences between controls and IHD: *p<0.05, †p<0.01, ‡p<0.001. [b]ANOVA tests between US-angina and S-angina : *p<0.05, †p<0.01. [c]ANOVA tests between US-angina and AMI: *p<0.05 . [d] ANOVA tests between S-angina and AMI: *p<0.05, †p<0.01. AMI - acute myocardial infarction

Table 6 - Effect of gender, age, diabetes mellitus, hypertension, and smoking on the mean (±SEM) plasma xanthine values (μM/L) in controls and patients with ischemic heart disease (IHD).

Parameters	Control	Stable angina	Unstable angina	AMI
Gender				
Women	0.40 ± 0.026	0.46 ± 0.026	0.51*[a] ± 0.022	0.60†[a] ± 0.052
Men	0.38 ± 0.093	0.40 ± 0.016	0.52*[a] ± 0.028	0.62‡[a] ± 0.022
Age (years)				
≤54years	0.39 ± 0.018	0.45 ± 0.016	0.53†[a] ± 0.034	0.65‡[a] ± 0.036
>54 years	0.39 ± 0.03	0.45 ± 0.026	0.49*[a] ± 0.01	0.57†[a] ± 0.024
Diabetes mellitus				
Diabetics	-	0.46 ± 0.022	0.50 ± 0.017	0.58*[b,*c]± 0.030
Non diabetics	-	0.44 ± 0.017	0.53 ± 0.056	0.64*[c] ± 0.026
Hypertension				
Hypertensives	-	0.45 ± 0.020	0.48 ± 0.024	0.58*[c] ± 0.024
Non-hypertensives	-	0.44 ± 0.018	0.54 ± 0.028	0.63*[b,†c] ± 0.032
Smoking				
Smokers	-	0.45 ± 0.040	0.50 ± 0.044	0.60*[b,*c]± 0.032
Non smokers	-	0.45 ± 0.026	0.52 ± 0.032	0.63*[b,*c] ± 0.022

Within group t-test for gender, age, diabetes, hypertensions, smoking: not significant. [a] analysis of variance (ANOVA) tests for gender and age differences between controls and IHD subgroups: *p<0.05, †p<0.01, ‡p<0.001. [b]ANOVA tests between US-angina and AMI: *p<0.05. [c]ANOVA tests between S-angina and AMI: *p<0.05, †p<0.01. AMI - acute myocardial infarction

Table 7 - Effect of gender, age, diabetes mellitus, hypertension, and smoking on the mean ±SEM plasma uric acid values (μM/L) in controls and patients with ischemic heart disease (IHD).

Parameters	Control	Stable angina	Unstable angina	AMI
Gender				
Women	226.8 ± 2.56	264.7 ± 26.80	405.2[†b] ± 77	379.2[†b] ± 27
Men	312.6[†a] ± 20.23	405.8[‡a,b] ± 12.5	368.3[† b] ± 20	405.9[‡b] ± 13
Age (years)				
≤54years	259.3 ± 22.61	346.9 [* b] ± 32.1	371.7[† b] ± 20	381.4[‡b] ± 17
>54 years	283.1 ± 268	324.4 ± 31	401.6[† b] ± 49	403.1[‡b] ± 15
Diabetes mellitus				
Diabetics	-	301.00 ± 30.40	396.90[*c] ± 38.70	405.80[†f] ± 16.7
Non diabetics	-	370.70 ± 26.20	375.60 ± 22.61	378.50 ± 15.50
Hypertension				
Hypertensives	-	364.70 ± 27.10	451.90[†c,*d] ± 40	393.9 [*c, f*] ± 22.00
Non-hypertensives	-	302.40 ± 34.50	321.70 ± 21.40	391.51[*c,f*] ± 12.50
Smoking				
Smokers	-	383.50[*c] ± 13.10	371.90 ± 24.10	387.70 ± 17.30
Non smokers	-	287.10 ± 33.10	401.80[†d] ± 50	396.90[†,f] ± 14.9

[a]Within group t-test: gender and age versus their respective counter-groups: [†]p<0.01, [‡]p<0.001
[b]ANOVA tests for gender and age differences between controls and IHD group: *p<0.05,†p<0.01 ,[‡]p<0.001.
[c]Within group t- test: diabetics, hypertensives, smokers versus their respective counter -groups: *p<0.05, †p<0.01.
[d]ANOVA tests between US- angina and S-angina: *p<0.05, †p<0.001.
[e]ANOVA tests between US- angina and AMI: *p<0.05. [f]ANOVA tests between S-angina and AMI: *p<0.05, †p<0.001.
AMI - acute myocardial infarction

compared to reference mean values. Furthermore, the mean ADO values rise in unstable angina and AMI patients in both age groups (≤54 and >54 years) with a marked significant increment in all age groups with AMI (p<0.001), although no significant difference was observed in the ADO values between the 2 different age groups presented with AMI or unstable angina. Testing the effect of diabetes, hypertension, and smoking on the ADO levels revealed a significant increase in the plasma mean values of ADO in diabetics with stable angina as compared to those of non-diabetics (p<0.05). Moreover, non-diabetics with unstable angina showed higher mean ADO values over non-diabetics with stable angina (p<0.05). Furthermore, diabetics with AMI showed a significant increase in the mean ADO values when compared to diabetics with stable angina or unstable angina (p<0.05). Also in **Table 3**, between groups, the ANOVA test revealed a significant rise in the mean values of ADO in hypertensives with unstable angina compared to stable angina (p<0.05). There was similar significant elevation of ADO levels in normotensives with AMI compared to the normotensives with stable angina (p<0.05). In the plasma of patients with unstable-angina or AMI, both INO and HYP (**Tables 4 & 5**) exhibited a statistically significant elevation, but the rises are more augmented in men with AMI (p<0.001) at age ≤54 years. The mean INO level is significantly raised in diabetic-AMI patients when compared to non-diabetic values (p<0.05). On the contrary, there was no significant influence of hypertension, and smoking on the mean plasma INO values in patients with IHD. But, hypertensives who suffered from unstable angina or AMI showed statistically significantly higher plasma INO over the values of hypertensives with stable angina (p<0.01). Diabetics with unstable angina have significantly higher mean HYP values over that of diabetics with stable angina (p<0.05). Moreover, the mean plasma HYP levels in hypertensives with unstable angina or AMI are significantly elevated above those of stable angina (p<0.01). Further increase in the mean plasma values is noted in patients with AMI compared to unstable angina (p<0.05). The mean plasma HYP level in non-smokers with AMI is observed to be significantly elevated (p<0.05) compared to nonsmokers with unstable angina. The effect of gender, age, diabetes mellitus, hypertension, or smoking on the mean ± SEM plasma level of XAN in both controls and patients with IHD is shown in **Table 6**. Within group and between groups, ANOVA tests reveal no significant gender or age differences in the mean values of XAN in the stable angina as compared to controls. Yet, the elevation in the XAN values in men and those patients below 54 years of age with AMI was significant (p<0.001) and exceeded the elevation recorded in men of similar age who suffered from unstable angina (p<0.01). Diabetic patients with AMI revealed a significant increase in the mean XAN level above those of diabetics who presented with unstable angina (p<0.05). In addition, the mean plasma XAN values were observed to be significantly raised in normotensives with AMI when compared to

those of the stable angina group ($p<0.01$). Furthermore, smokers who experienced AMI or unstable angina have significantly higher XAN values as compared to smokers with stable angina ($p<0.05$). Within IHD group ANOVA test revealed no significant difference between smoker and non-smoker values. **Table 7** reflects the effect of gender, age, diabetes mellitus, hypertension and smoking on the mean plasma UA values in the controls and patients with IHD. The mean reference plasma UA level in men was significantly higher ($p<0.01$) than that of normal women values. Patients with diabetes mellitus, hypertensive who suffer from unstable angina as well as smokers with stable angina have statistically significant rise in the mean plasma UA values above normotensives ($p<0.01$), and non-smoker values ($p<0.05$). Moreover, between groups ANOVA tests pinpoint significant elevation in the mean plasma UA values in non-smokers with unstable angina or AMI as compared to non-smokers with stable angina ($p<0.01$).

Discussion. Purine-mediated free radical production plays a crucial role in reperfusion injury.[5,14] During severe ischemia, ATP consumption more than limited ATP production by anaerobic glycolysis, is a key factor affecting recovery on subsequent reperfusion. In contrast to lactate efflux, purine and nor adrenaline release are useful markers of ischemic and reperfusion damage.[15] The large stores of ATP in the myocardium are constantly depleted during contraction and replenished by oxidative phosphorylation. With ischemia, the re-synthesis of ATP decreases and its nucleoside precursors accumulate. The rapid decrease of oxygen and substrate availability to ischemic tissues induces the inhibition of oxidative metabolism, which is fundamental for the cellular energy requirement. This is reflected by the sudden depletion of ATP, which is sequentially dephosphorylated to ADO (partly deaminated to INO) and up to the free purines (HYP and XAN) thus, leading to an accumulation of these compounds inside the tissue.[16] The release of these nucleosides may therefore provide an additional biochemical index of current or prior ischemia.[17] In the present study, the elevation of the plasma ADO values in IHD may be attributed to many factors, apart from ischemia, such as medication. A significant number of our patients use the drug dipyridamole, which acts as a potent inhibitor to the enzyme ADO deaminase (ADA). Furthermore, it inhibits platelets aggregation and their liberation of ADP during the release reaction. This latter action might be of particular importance since the degradation of ADP by cellular ectonucleotidases could give rise to the formation of ADO during sampling processes.[18] This fact is stressed by the work of Sollevi[19] who reported that dipyridamole (after pacing induced ischemia) induces the release of ADO from human heart and causes a rise in the plasma ADO level. Furthermore, the extra cellular adenine nucleotide could be derived from aged erythrocytes and from platelets.[20] Moreover, Cunha et al[21] reported an increase in the free fatty acid levels and a concomitant decrease in the density and potency of A1 receptors for ADO in rats with aging, and they speculated that this may contribute to the decreased adaptability of the neuromodulation to different firing conditions in aged rats. Hinschen et al[22] reported that ADO mediates nitric oxide-dependent and independent coronary and aortic relaxation in rats. Moreover, they noted that age reduces the NO-dependent and NO-independent ADO response in addition to decline in ADO receptor transduction maximum. Furthermore, Burnstock[23] found that ATP released from endothelial cells during changes in flow (shear stress) or hypoxia acts on P2Y receptors in endothelial cells to release NO, which results in relaxation. Adenosine, following breakdown of extra cellular ATP, produces vasodilatation via smooth muscle P1 receptors. Heaps and Bowles[24] assessed the influence of gender on the ADO-mediated relaxation of coronary arterioles. They proposed that the underlying mechanism of relaxation is directly related to sex hormones. They pointed out that both estrogen and testosterone activate potassium (K+) channels to produce vascular smooth muscle relaxation. Moreover, they reported that the arterioles of female pigs utilize an additional vasodilatory pathway to compensate for the lack of K+-channel contribution. Gassar et al[25] investigated the effects of hyperglycemia-mediated impairment in the nucleoside uptake on the actions of endogenous ADO in rat. They reported that in control tissue under conditions of anoxia/aglycemia, the rise in the extra cellular ADO concentration resulted in a complete inhibition of synaptic activity in approximately 2 minutes. These changes could be prevented by insulin treatment. Furthermore, the ADO A1 receptor antagonist prevented the anoxia/aglycemia-mediated inhibition and abolished the differences in the electrophysiological responses between controls and diabetic tissue. Pretreatment with 50 mg of ADO decreases the incidence of myonecrosis after nonurgent percutaneous coronary intervention.[26] The impairment of nucleoside uptake by chronic hyperglycemia results in the potentiation of the modulatory actions of endogenous ADO in the central nervous system.[25] Abd-Elfattah[14] reported that more than 90% of ATP degraded during ischemia is retained inside the cardiomyocytes as INO. During reperfusion and in the presence of molecular oxygen, INO is released via nucleoside transporters and rapidly converted to HYP

and XAN leading to the generation of super oxide anion radicals.[27] The higher expected INO values reported in our study may be attributed to the great INO permeability of human erythrocytes as a source of error that is generated from a minute hemolysis or due to the delay in the separation of serum from RBCs.[28] Olsson[29] observed an increase in both ADO and INO during coronary occlusion in an intact dog heart. Furthermore, they showed that the ADO level appeared to be related more closely to the number of beats occurring during occlusion, and they suggested that ADO production is somehow linked to the heart metabolism as the heart rate is an important determinant of myocardial oxygen consumption. Kugler et al[30] reported that in patients with IHD during pacing-induced angina, the INO level was significantly increased from 535±18nM/L at rest to 1235±800 nM/L during angina, whereas the HYP level was not significantly increased from 1000±76nM/L to 1235±800nM/L during angina. Edlund et al[31] observed that large amounts of HYP were released during angina and that during atrial pacing the arterial and coronary sinus concentration of INO remained low. The increased release of INO may be due to the high ADO deaminase activity and low activity of the nucleoside phosphorylase enzyme, which is mainly localized in the endothelium of coronary vessels. Kock et al[32] recorded a significant increase in the serum HYP, XAN, UA, and allantoin in patients with AMI but not in stable angina or control subjects indicating a significant metabolic involvement of XAN oxidase and therefore its possible role in the development of tissue damage in the post ischemic phase due to oxygen radicals generated by the XAN oxidase. Vlessis et al[33] recorded an elevation in the level of HYP in the coronary sinus to 7 folds (p<0.001) and XAN to 2 folds (p<0.02) after reperfusion, and they speculated that the rise in coronary sinus XAN concentration provides an evidence for the HYP degradation by XAN oxidase during the reperfusion period. Fox et al[17] recorded the appearance of ADO in the coronary sinus blood from patients with IHD during pacing-induced ischemia with no difference between arterial and coronary sinus concentration of INO. However, the breakdown products of INO (HYP) were present in a high concentration, this indicates an ischemia-induced facilitated breakdown of energy-rich adenine nucleotides in the heart with subsequent release of ADO, which further metabolized to HYP.[31] Furthermore, the urinary excretion of XAN and HYP is greatly reduced in renal failure, which reflects an additional cause beyond the high serum values in such patients.[40] In 1994, Lazzarino et al[34] found that the concentrations of ADO, INO, XAN, and UA were increased up to 2.8 folds after aortic cross clamping and then gradually decreased after de-clamping of the aorta. Kock et al[32] reported that an increase in serum XAN content in patients after myocardial infarction indicates a significant metabolic involvement of XAN oxidoreductase in acute infarction, and its possible role in the development of tissue damage in the post ischemic phase. The UA level in the present study was nearly similar to those of Al-Mudares[36] and Al-Zamely[37] who reported a mean UA level of 351.6 µM/L and 343.5µM/L, and is higher than those of Zakaria et al,[38] who obtained a mean UA value of 191±42.2 µM/L of range between 110-292 µM/L. The role of serum uric acid (SUA) as an independent risk factor for the morbidity and mortality from cardiovascular disease and stroke remains controversial. In a large prospective cohort study on 83683 Australian men, SUA was reported to be independently related to mortality from congestive heart failure and stroke. Although increased SUA is not necessarily a causal risk factor, it is still of clinical significance in disease monitoring and intervention.[39] Hyperuricemia has been found to be associated with obesity and insulin resistance, and consequently with type 2 diabetes mellitus.[10] The relation between UA and diastolic blood pressure was described by Puig[40] who reported an increase in blood urate level in around a quarter of patients with hypertension. This observation is confirmed in our study by finding a significant positive correlation between the mean diastolic blood pressure and plasma UA (r=0.39, p<0.036) and also stressed by the study of Voelkel and Reeves[41] who noticed a strong correlation between UA and the right arterial pressure (r=0.47, p<0.001) in patients with primary pulmonary hypertension. In the present study, we observed that smokers who developed AMI had higher serum UA values compared to smokers with stable angina. This is attributed to the fact that chronic smoking reduces the delivery of oxygen to the heart and decreases ATP production, which leads to an increase in serum UA as an end product of ATP depletion. Moreover, cigarette smoke increases platelet's activity, accelerates atherosclerotic lesions, and enhances tissue damage following ischemia or myocardial ischemia.[42] Serum UA is high in both genders who were taking antihypertensive drugs and diuretics.[43] The male mean UA serum value was found to be higher than the female mean UA value, due to the increased body mass index or high protein intake.[44] Although there is a limited extensive work on the relation of the anatomical location of myocardial wall infarction and these biochemical parameters yet, Zmudka et al[45] observed that the amount of the purine degradation products released during ischemia and reperfusion in rats correlates with the infarct size and is inversely

proportional to the area of viable myocardium. When Backstrom et al[46] induced AMI in 20 anesthetized pigs by the occlusion of the left anterior descending artery, they noticed a positive correlation between the infarct size and the myocardial outflow of amino acids and purines as monitored by the intravasal micro dialysis in the myocardial venous outflow during ischemia and reperfusion. Moens et al,[47] reported that myocardial high-energy phosphates ATP and ADP reduced by ischemia in rat controls were better preserved by folic acid (FA) pretreatment. Basal oxypurines (XAN, HYP, and urate) rose with FA pretreatment, but increased less during ischemia than in controls. After reperfusion, FA-treated hearts had smaller infarcts and less contraction band necrosis.[47]

Mention should be made of the limitations of this study. First, the measurement was conducted on patients who were already receiving medications that affect some purine catabolites such as digitalis, dipyridamole, diuretics, aspirin, and so forth. Secondly, it was very difficult to come across patients who attend the cardiology unit at very early hours from the feeling of chest pain especially if we pinpoint the fact that measurement of some purines such as ADO, are technically difficult because of short half life in the plasma.[48] Third, exclusion of AMI patients who develop heart failure, especially those with extensive wall infarction has led to the reduction in the sample number, which causes the statistical insignificant differences.

In conclusion, during ischemia the continuous generation of free purines (HYP, XAN, and UA) inside the myocytes leads to accumulation of these compounds inside the myocardium.[35] However, the myocardial cell membrane is permeable to these nucleosides and during reperfusion they are washed out of the ischemic myocardium into the coronary sinus effluent and are detectable in the arterial and coronary venous blood.[49] So, pretreatment with low dose of purines (ADO and INO) or oxypurines (XANs) may be effective in eliminating the deleterious effect of the oxygen derived radicals during the reperfusion stage and to improve the regional ventricular function after coronary artery occlusion.

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References

1. Hearse DJ. Myocardial ischaemia: can we agree on a definition for the 21st century? ***Cardiovasc Res*** 1994; 28: 1737-1744:
2. Braunwald E. Biomarkers in heart failure. ***N Engl J Med*** 2008; 358: 2148-2159.
3. Pepine CJ. Prognostic implications of silent myocardial ischemia. ***N Engl J Med*** 1996; 334: 113-114.
4. Lu J, Zang WJ, Yu XJ, Chen LN, Zhang CH, Jia B. et al. Effects of ischaemia-mimetic factors on isolated rat ventricular myocytes. ***Exp Physiol*** 2005; 90: 497-505.
5. Zmudka K, Zalewski J, Dubiel J, Gajos G, Legutko J, Dudek D, et al. Ischemic and reperfusive release of the endogenous purines and its influence on the myocardial viability during beta-adrenergic blockade. ***Pol J Pharmacol*** 2001; 53: 271-282.
6. Vinten-Johansen J, Zhao ZQ, Corvera JS, Morris CD, Budde JM, Thourani VH, et al. Adenosine in myocardial protection in on-pump and off-pump cardiac surgery. ***Ann Thorac Surg*** 2003; 75: S691-S699.
7. Devous MD Sr, Lewandowski ED. Inosine preserves ATP during ischemia and enhances recovery during reperfusion. ***Am J Physiol*** 1987; 253 (5 Pt 2): H1224-H1233.
8. Chambers DE, Parks DA, Patterson G, Roy R, McCord JM, Yoshida S, et al. Xanthine oxidase as a source of free radical damage in myocardial ischemia. ***J Mol Cell Cardiol*** 1985; 17: 145-152.
9. Hayden MR, Tyagi SC. Uric acid: A new look at an old risk marker for cardiovascular disease, metabolic syndrome, and type 2 diabetes mellitus: The urate redox shuttle. ***Nutr Metab (Lond)*** 2004; 1: 10.
10. Koenig W, Meisinger C. Uric acid, type 2 diabetes, and cardiovascular diseases: fueling the common soil hypothesis? ***Clin Chem*** 2008; 54: 231-233.
11. Bloomfield P, Bradburg A, Grubb NR, Newby DE. Cardiovascular disease. In: Boon N, Colledge N, Walker B, Hunter JAA, editors. Davidson's Principles and Practice of Medicine. 20th ed. London (UK): Elsevier; 2006. p. 581-600.
12. Burtis CA, Ashwood ER, Bruns DE, editor. Teitz Fundamentals of Clinical Chemistry. 6th ed. London (UK): Elsevier; 2008.
13. Feng JD, Yeung PK. A simple high-performance liquid chromatography assay for simultaneous measurement of adenosine, guanosine, and the oxypurine metabolites in plasma. ***Ther Drug Monit*** 2000; 22: 177-183.
14. Abd-Elfattah AS, Jessen ME, Lekven J, Doherty NE 3rd, Brunsting LA, Wechsler AS. Myocardial reperfusion injury. Role of myocardial hypoxanthine and xanthine in free radical-mediated reperfusion injury. ***Circulation*** 1988; 78 (5 Pt 2): III224-III35.
15. Cargnoni A, Ceconi C, Curello S, Benigno M, de Jong JW, Ferrari R. Relation between energy metabolism, glycolysis, noradrenaline release and duration of ischemia. ***Mol Cell Biochem*** 1996; 160-161: 187-194.
16. Lazzarino G, Vagnozzi R, Tavazzi B, Pastore FS, Di Pierro D, et al. MDA, oxypurines, and nucleosides relate to reperfusion in short-term incomplete cerebral ischemia in the rat. ***Free Radic Biol Med*** 1992; 13: 489-498.
17. Fox AC, Reed GE, Meilman H, Silk BB. Release of nucleosides from canine and human hearts as an index of prior ischemia. ***Am J Cardiol*** 1979; 43: 52-58.
18. Ontyd J, Schrader J. Measurement of adenosine, inosine, and hypoxanthine in human plasma. ***J Chromatogr*** 1984; 307: 404-409.
19. Sollevi A. Cardiovascular effects of adenosine in man; possible clinical implications. ***Prog Neurobiol*** 1986; 27: 319-349.
20. Möser GH, Schrader J, Deussen A. Turnover of adenosine in plasma of human and dog blood. ***Am J Physiol*** 1989; 256 (4 Pt 1): C799-C806.
21. Cunha RA, Constantino MD, Fonseca E, Ribeiro JA. Age-dependent decrease in adenosine A1 receptor binding sites in the rat brain. Effect of cis unsaturated free fatty acids. ***Eur J Biochem*** 2001; 268: 2939-2947.

22. Hinschen AK, Rose'Meyer RB, Headrick JP. Age-related changes in adenosine-mediated relaxation of coronary and aortic smooth muscle. ***Am J Physiol Heart Circ Physiol*** 2001; 280: H2380-H2389.
23. Burnstock G. Historical review: ATP as a neurotransmitter. ***Trends Pharmacol Sci*** 2006; 27: 166-176.
24. Heaps CL, Bowles DK. Gender-specific K(+)-channel contribution to adenosine-induced relaxation in coronary arterioles. ***J Appl Physiol*** 2002; 92: 550-558.
25. Cassar M, Jones MG, Szatkowski M. Reduced adenosine uptake accelerates ischaemic block of population spikes in hippocampal slices from streptozotocin-treated diabetic rats. ***Eur J Neurosci*** 1998; 10: 239-245.
26. Lee CH, Low A, Tai BC, Co M, Chan MY, Lim J, Lim YT, et al. Pretreatment with intracoronary adenosine reduces the incidence of myonecrosis after non-urgent percutaneous coronary intervention: a prospective randomized study. ***Eur Heart J*** 2007; 28: 19-25.
27. Lefer DJ, Granger DN. Oxidative stress and cardiac disease. ***Am J Med*** 2000; 109: 315-323.
28. Duhm J. Inosine permeability and purine nucleoside phosphorylase activity as limiting factors for the synthesis of 2,3-diphosphoglycerate from inosine, pyruvate, and inorganic phosphate in erythrocytes of various mammalian species. ***Biochim Biophys Acta*** 1974; 343: 89-100.
29. Olsson RA. Changes in coronary venous inosine concentration and myocardial wall thickening during regional ischemia in the pig. ***Circ Res*** 1970; 26: 301-306.
30. Kugler G. Myocardial release of inosine, hypoxanthine and lactate during pacing-induced angina in humans with coronary artery disease. ***Eur J Cardiol*** 1979; 9: 227-240.
31. Edlund A, Berglund B, van Dorne D, Kaijser L, Nowak J, Patrono C, et al. Coronary flow regulation in patients with ischemic heart disease: release of purines and prostacyclin and the effect of inhibitors of prostaglandin formation. ***Circulation*** 1985; 71: 1113-1120.
32. Kock R, Delvoux B, Sigmund M, Greiling H. A comparative study of the concentrations of hypoxanthine, xanthine, uric acid and allantoin in the peripheral blood of normals and patients with acute myocardial infarction and other ischaemic diseases. ***Eur J Clin Chem Clin Biochem*** 1994; 32: 837-842.
33. Vlessis AA, Ott G, Cobanoglu A. Purine efflux from transplanted human cardiac allografts. Correlation with graft function. ***J Thorac Cardiovasc Surg*** 1994; 107: 482-486.
34. McBurney A, Gibson T. Reverse phase partition HPLC for determination of plasma purines and pyrimidines in subjects with gout and renal failure. ***Clin Chim Acta*** 1980; 102: 19-28.
35. Lazzarino G, Raatikainen P, Nuutinen M, Nissinen J, Tavazzi B, Di Pierro D, et al. Myocardial release of malondialdehyde and purine compounds during coronary bypass surgery. ***Circulation*** 1994; 90: 291-297.
36. Al-Mudares KMS. The relation of oxygen free radicals to acute myocardial infarction complications (Thesis). Iraq: Saddam College of Medicine; 1999
37. Al-Zamely OMY. Ischemic heart disease via oxidative hypothesis. (Thesis). Iraq: Mustansiryia, College of Medicine; 2001.
38. Zakaria M, Brown PR, Farnes MP, Barker BE. HPLC analysis of aromatic amino acids, nucleosides, and bases in plasma of acute lymphocytic leukemia on chemotherapy. ***Clin Chim Acta*** 1982; 126: 69-80.
39. Strasak A, Ruttmann E, Brant L, Kelleher C, Klenk J, Concin H, et al. Serum uric acid and risk of cardiovascular mortality: a prospective long-term study of 83,683 Austrian men. ***Clin Chem*** 2008; 54: 273-284.
40. Puig JG, Mateos F, Buño A, Ortega R, Rodriguez F, Dal-Ré R. Effect of eprosartan and losartan on uric acid metabolism in patients with essential hypertension. ***J Hypertens*** 1999; 17: 1033-1039.
41. Voelkel NF, Reeves JT. Primary pulmonary hypertension. In: Moser KM, ediotr. Pulmonary vascular. Volume 14. New York (NY): Marcel Dekker; 1979. p. 573-628.
42. Glantz SA, Parmley WW. Passive smoking and heart disease. Mechanisms and risk. ***JAMA*** 1995; 273: 1047-1053.
43. Brand FN, McGee DL, Kannel WB, Stokes J 3rd, Castelli WP. Hyperuricemia as a risk factor of coronary heart disease: The Framingham Study. ***Am J Epidemiol*** 1985; 121: 11-18.
44. Klein BE, Klein R, Lee KE. Components of the metabolic syndrome and risk of cardiovascular disease and diabetes in Beaver Dam. ***Diabetes Care*** 2002; 25: 1790-1794.
45. Zmudka K, Zalewski J, Dubiel J, Gajos G, Legutko J, Dudek D, et al. Ischemic and reperfusive release of the endogenous purines and its influence on the myocardial viability during beta-adrenergic blockade. ***Pol J Pharmacol*** 2001; 53: 271-282.
46. Bäckström T, Goiny M, Lockowandt U, Liska J, Franco-Cereceda A. Cardiac outflow of amino acids and purines during myocardial ischemia and reperfusion. ***J Appl Physiol*** 2003; 94: 1122-1128.
47. Moens AL, Champion HC, Claeys MJ, Tavazzi B, Kaminski PM, Wolin MS, et al. High-dose folic acid pretreatment blunts cardiac dysfunction during ischemia coupled to maintenance of high-energy phosphates and reduces postreperfusion injury. ***Circulation*** 2008; 117: 1810-1819.
48. Saito H, Nishimura M, Shibuya E, Makita H, Tsujino I, Miyamoto K, et al. Tissue hypoxia in sleep apnea syndrome assessed by uric acid and adenosine. ***Chest*** 2002; 122: 1686-1694.
49. Jennings RB, Reimer KA, Hill ML, Mayer SE. Total ischemia in dog hearts, in vitro. 1. Comparison of high energy phosphate production, utilization, and depletion, and of adenine nucleotide catabolism in total ischemia in vitro vs. severe ischemia in vivo. ***Circ Res*** 1981; 49: 892-900.