Chapter 7

Is Hyperuricemia a Risk Factor to Cardiovascular Disease?

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Additional information is available at the end of the chapter

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1. Introduction

Uric acid is a weak acid distributed throughout the extracellular fluid compartments (Emmerson, 1996). The normal blood uric acid level in humans is approximately 4 mg/dl (0.24 mmol/l) (Ganong, 2005). Uric acid is the end product of purine degradation. Purines are degraded ultimately to uric acid through the action of the enzyme xanthine oxidase that converts xanthine to urate (Mc Lean, 2003). In most mammals, the liver enzyme uricase (urate oxidase) is responsible for further metabolism of uric acid to allantoin, which is more soluble waste product. However, humans lack the enzyme uricase, resulting in higher blood uric acid levels (Hediger et al., 2005). They might provide humans a survival advantage over the other primates because of the function of uric acid as antioxidant (Mc Lean, 2003).). For an individual, urate concentration is determined by the balance between the rate of purine metaboloism, both endogenous and exogenous, and the efficiency of renal clearance. Alteration in this balance may account for hyperuricemia. In the majority (90%) of patients with primary gout, hyperuricemia results from relative renal undersecretion, whereas in 10% of patients, there is overproduction of endogenous uric acid (Fam, 2002).

Elevated serum uric acid, besides its documented link to gouty arthritis, has been reported to be closely-associated with the metabolic syndrome and, as well, to be a correlate of the development and progression of cardiovascular diseases (Baker et al., 2005), though the role of uric acid in this respect is still unclear. Several possible pathological mechanisms linking hyperuricemia to cardiovascular disease were suggested; including the deleterious effects of elevated uric acid on endothelial dysfunction, oxidative metabolism, as well as platelet adhesiveness, hemorheology and aggregation (Hoieggen et al., 2003). However, no enough or definite experimental data exist concerning the association of hyperuricemia with the different cellular elements of blood. The aim of this work was to investigate the effects of elevated serum

uric acid levels on the physiology of the different cellular blood elements in rats and their link to cardiovascular ailment.

2. Methods

This study was performed on 58 albino rats, of both sexes, weighing 180- 250g. Rats included in the present study were divided into 2 main groups: hyperuricemic group (group I) and normouricemic control group (group II). Hyperuricemia was achieved by using the uricase inhibitor oxonic acid (oxonic acid, potassium salt, supplied by Acros Organics, Geel, Belgium), with concomitant supplementation of diet with 3% uric acid. Oxonic acid was administered orally for 5 days, 2 and 4 weeks by gavage in a dose of 750 mg/kg body weight (Khosla et al., 2005), dissolved in 0.25% methylcellulose. Concomitant supplementation of diet with uric acid was done to obtain better and maintained hyperuricemic response (Mazzali et al., 2001). The control groups were received the solvent orally for 5 days, 2 and 4 weeks. Studied rats were allocated into the following groups and subgroups: Group I: Hyperuricemic rats, that received the oxonic/uric acid regimen. Rats in this group were further subdivided into 3 subgroups according to the duration of hyperuricemia: Group Ia: Five-days hyperuricemic rats (n=10), receiving the oxonic/uric acid regimen for 5 consecutive days. Group Ib: Two-weeks hyperuricemic rats (n=11), receiving the regimen 6 days/week for 2 weeks. Group Ic: Four-weeks hyperuricemic rats (n=10), receiving the regimen 6 days /week for 4 weeks. Group II: Normouricemic control rats, that received methylcellulose, the solvent for oxonic acid, orally by gavage. Rats in this group were further subdivided into 3 subgroups, matching the hyperuricemic subgroups:

Group IIa: Five-days control rats (n=8), receiving the solvent for five consecutive days. Group IIb: Two-weeks control rats (n=8), receiving the solvent 6 days/week for 2 weeks. Group IIc: Four-weeks control rats (n=11), receiving the solvent 6 days/week for 4 weeks.

Experimental procedure: At the end of the experimental period, overnight fasted rats were weighed and anaesthetized with intraperitoneal injection of sodium thiopental (40 mg/kg body weight). One ml of blood was drawn into a tube containing EDTA for assessment of complete blood picture. Three ml of blood was collected in a chilled plastic tube containing sodium citrate 3.8%, gently mixed for assessment of platelet aggregation. Another blood samples were collected in chilled plastic tubes containing sodium citrate 3.8gm/100 ml (9 volumes of blood to 1 volume of sodium citrate) and gently shaken. These blood samples were used for study of platelet aggregation. The citrated blood was centrifuged at 1500 r.p.m. for 5 min. The supernatant platelet rich plasma (PRP) was pipetted into clean plastic tubes. The remaining blood sample was centrifuged at 10,000 r.p.m. for 10 min. to prepare platelet poor plasma (PPP). Standard PRP: the number of platelets in PRP was counted using coulter T-660 counter. The platelet number was adjusted to a standardized number of 3×10^5 platelet per μl by dilution with autologus platelet poor plasma.

Aggregation study: platelet aggregation was performed using Chrono-Log automatic aggregometer (model 540-VC, Chrono-Log Corp, Harvertown, USA) coupled with computer and

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printer. ADP as an aggregating agent was used at a final concentration of 10 uM. The maximum aggregation was recorded after 3 min. Platelet aggregation was tested by the turbidimetric technique, according to the method of Mustard et al. (1964). Platelet aggregation was induced in stirred platelet-rich plasma (PRP) by addition of the aggregating agent ADP (Park com). The platelet count in PRP was adjusted to a standardized number of 3x105 platelet/μl by dilution with autologus platelet poor plasma to obtain standard PRP. Platelet aggregation was performed by the use of a chrono-Log automatic aggregometer (model 540, chrono-Log Corporation, Harvertown, USA), coupled with computer and printer.

Enzymatic determination of plasma uric acid was carried out according to the method of Barhan and Trinder (1972). Nitrate concentration in plasma was estimated according to the method described by Bories and Bories (1995). C-reactive protein (CRP) was determined qualitatively by the use of latex slide test, described by Singer et al. (1957).

3. Results

Administration of oxonic/uric acid regimen for 5 days, 2 and 4 weeks resulted in a highly significant hyperuricemic response, being less marked in the 4-weeks treated group. This observation could be explained by increased urinary uric acid excretion in the later group. Hediger et al. (2005) demonstrated increased mRNA transcription of the urate transporters ($URAT_1$) in association with hyperuricemia. No significant changes were found as regard erythrocyte parameters, platelet count or mean platelet volume between hyperuricemic and control groups.

As regards the leucocyte changes accompanying hyperuricemia, the neutrophil% was significantly increased and lymphocyte % was significantly decreased compared to control values despite the non-significant changes in the total leucocytic count. Neutrophils were speculated to liberate a potent activation signal after interaction with monosodium urate crystals, and that this activation can further stimulate surrounding neutrophils and contribute to amplification of the inflammatory response, with the redox pathways being implicated in these reactions (Desaulniers et al., 2006). Further, C-reactive protein, the systemic inflammatory marker, was markedly increased in the 2- and 4-weeks hyperuricemic rats compared to controls. Uric acid was reported to induce expression of CRP in vascular endothelial and smooth muscle cells, which was proposed to provide a pathogenic link to explain the association of the systemic inflammatory response and elevated uric acid in patients with cardiovascular disease and diabetes.(Kang et al., 2005; Saito et al., 2003). The increased neutrophil %, and the significant positive correlation observed between uric acid level and neutrophil %, together with the increased CRP, encountered in the present study, support the previous findings of Ruggiero et al.(2006) pointing to significant and independent association of uric acid level with neutrophil count and CRP level. On the other hand, the lymphocyte % was significantly reduced in all the hyperuricemic groups. The encountered lymphocytopenia could be attributed to the increased free radical burden in hyperuricemia, leading to increased lymphocyte apoptosis. Uric acid has been demonstrated to act in some instances as prooxidant, generating free radicals (Patterson et al., 2003).

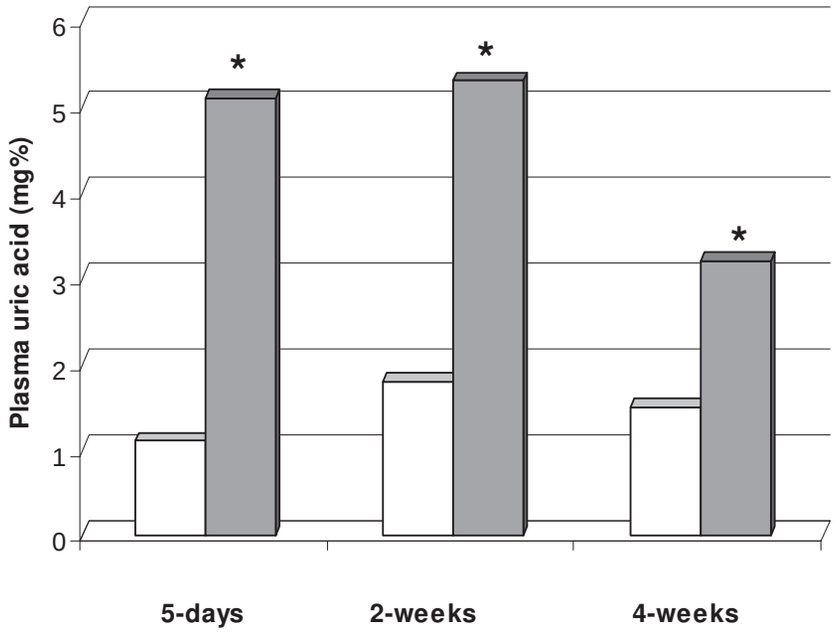

Figure 1. changes in plasma uric acid in the different hyperuricemic groups and their matching control groups

With regard to platelet changes associated with hyperuricemia, enhanced ADP-induced platelet aggregation was encountered in all the hyperuricemic groups compared to controls, the effect being more marked in the 5-days and 2-weeks groups, showing higher uric acid levels. Moreover, a significant positive correlation between plasma uric acid and platelet aggregation was demonstrated in all the tested groups of rats. The observed enhancement of platelet aggregation is in accordance with previous reports (Alderman and Aiyer, 2004).

The enhanced platelet aggregation demonstrated in the present study could be explained by many mechanisms. Urate crystals were reported to stimulate arachidonic acid metabolism in platelets.(Serhan et al., 1984) Moreover, neutrophilia demonstrated in the present study might contribute to platelet hyperaggregability, as neutrophils were reported to be potent inducers of platelet Ca^{2+} flux, aggregation and secretion. (Faint, 1992). Furthermore, the observed lymphocytopenia could play a role in the increased platelet activity. In 1987, Wu et al. proposed lymphocytes to possess PGI_2 synthase activity which is capable of converting platelet-derived PGH_2 into PGI_2 that is sufficient to inhibit platelet function. Therefore, the encountered decrease in lymphocytes could provide an additional explanation for the enhanced platelet aggregability.

The observed decrease in plasma nitrate level in the 4-weeks hyperuricemic rats, together with the significant negative correlation between plasma levels of uric acid and nitrate demonstrated in this group, reflect possible lowering of nitric oxide (NO) bioavailability with prolongation of hyperuricemia. Reduced NO production in association with hyperuricemia has been reported by several investigators.(Waring and Esmail, 2005) The reduced NO, together with

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the inflammatory response, could imply increased risk of cardiovascular pathology in hyperuricemic subjects by promoting development of endothelial dysfunction.

In conclusion, the neutrophil and platelet activation, known predisposing factors to thrombosis, together with increased CRP production and reduced NO production, might share in causing the hyperuricemia-associated endothelial dysfunction and atherosclerotic plaque formation. Therefore, it could be recommended that physicians should be aware of the role of elevated uric acid in inducing cardiovascular insult, and that individuals suffering from hyperuricemia should be advised to have a strict follow-up for their platelet function, which could participate in the cardiovascular pathology.

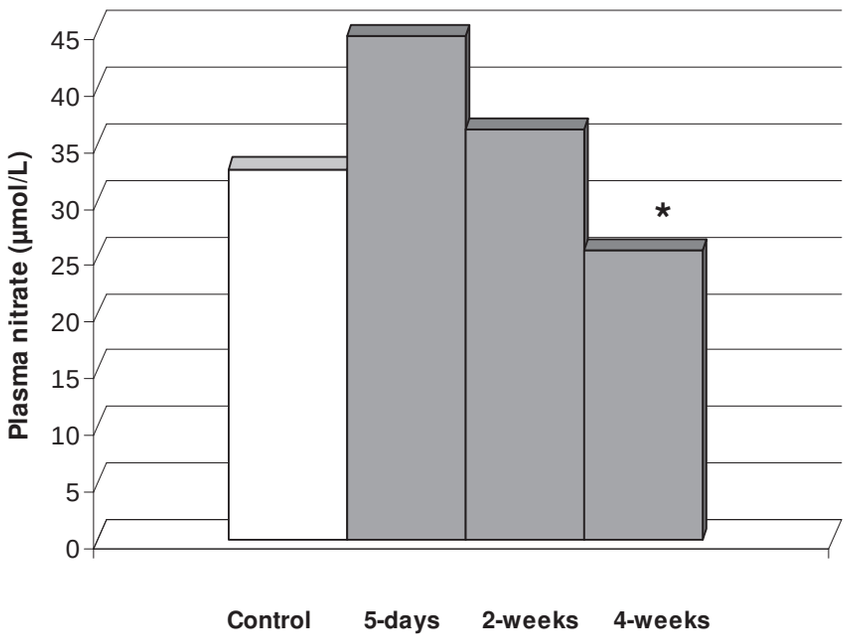

Figure 2. changes in plasma nitrate in the normouricemic and the different hyperuricemic groups

Duration	5-days hyperuricemia			2-weeks hyperuricemia			4-weeks hyperuricemia		
Parameter	Control rats	Hyper-uricemic rats	P	Control rats	Hyper-uricemic rats	P	Control rats	Hyper-uricemic rats	P
TLC ($x10^3/\mu l$)	3.2±0.38 (7)	3.0±0.56 (9)	NS	2.8±0.39 (7)	3.2±0.59 (9)	NS	3.7±0.52 (9)	3.9±0.76 (10)	NS
Neutrophil (%)	31.6±2.93 (7)	53.1±3.87 (9)	<0.001	34.4±4.25 (7)	56.9±6.92 (9)	<0.05	35.0±7.25 (9)	58±6.42 (10)	<0.05
Lymphocyte %	67.6±3.37 (7)	46.2±4.00 (9)	<0.01	64.3±4.12 (7)	41.0±7.17 (9)	<0.05	64.6±7.23 (9)	41.5±6.33 (10)	<0.05

Table 1. Results of the changes in leucocyte parameters in the different studied groups **P**: Significance of difference from matched control rats calculated by Student's "t"test for unpaired data. **NS**: Not significant.

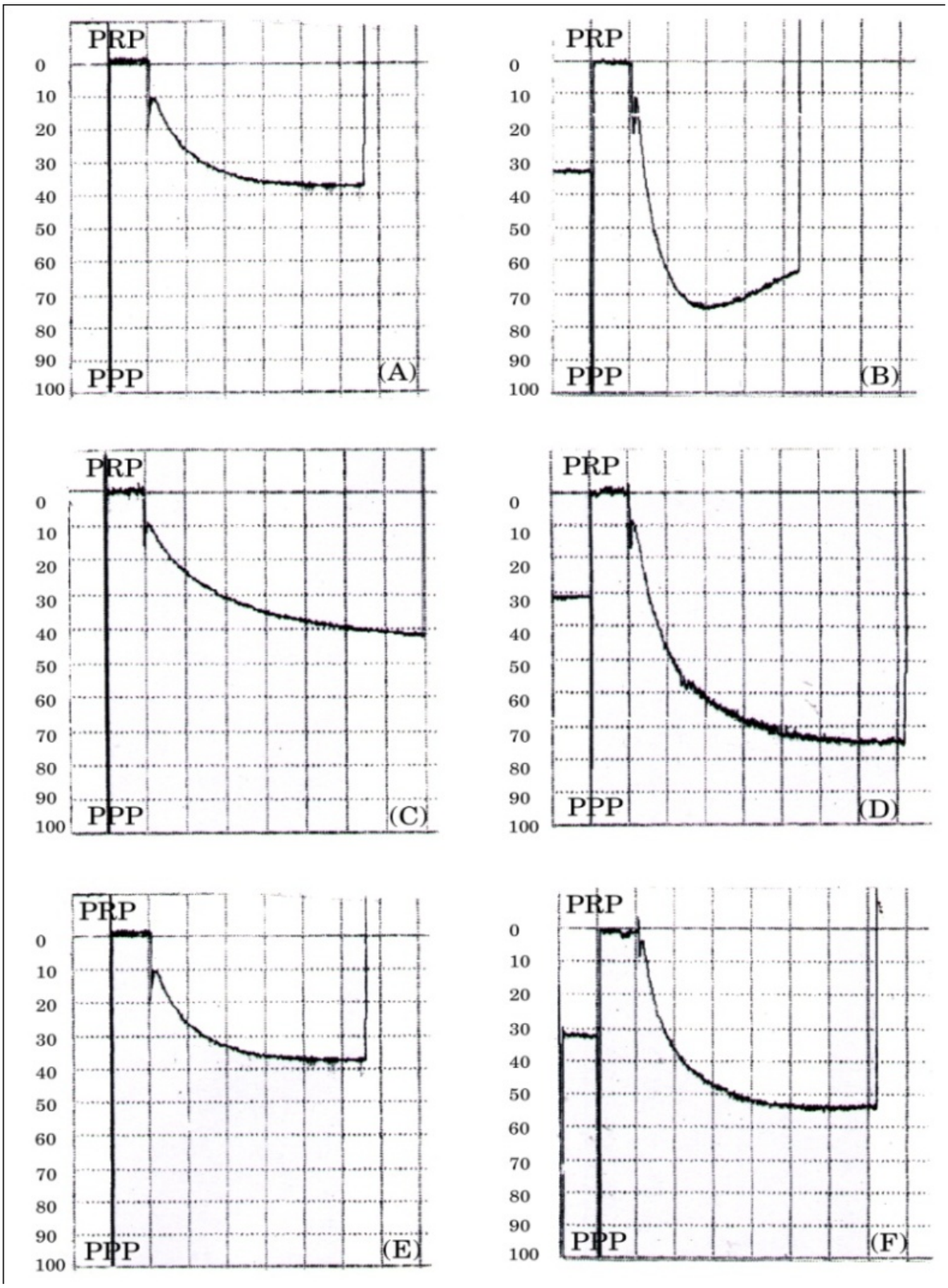

Figure 3. Tracing of ADP-induced platelet aggregation in the different studied groups; 5 days normouricemic rats (A), hyperuricemic rats for 5 days (B), normouricemic rats for 2 weeks (C), hyperuricemic rats for 2 weeks (D), normouricemic rats for 4 weeks (E) and hyperuricemic rats for 4 weeks (F).

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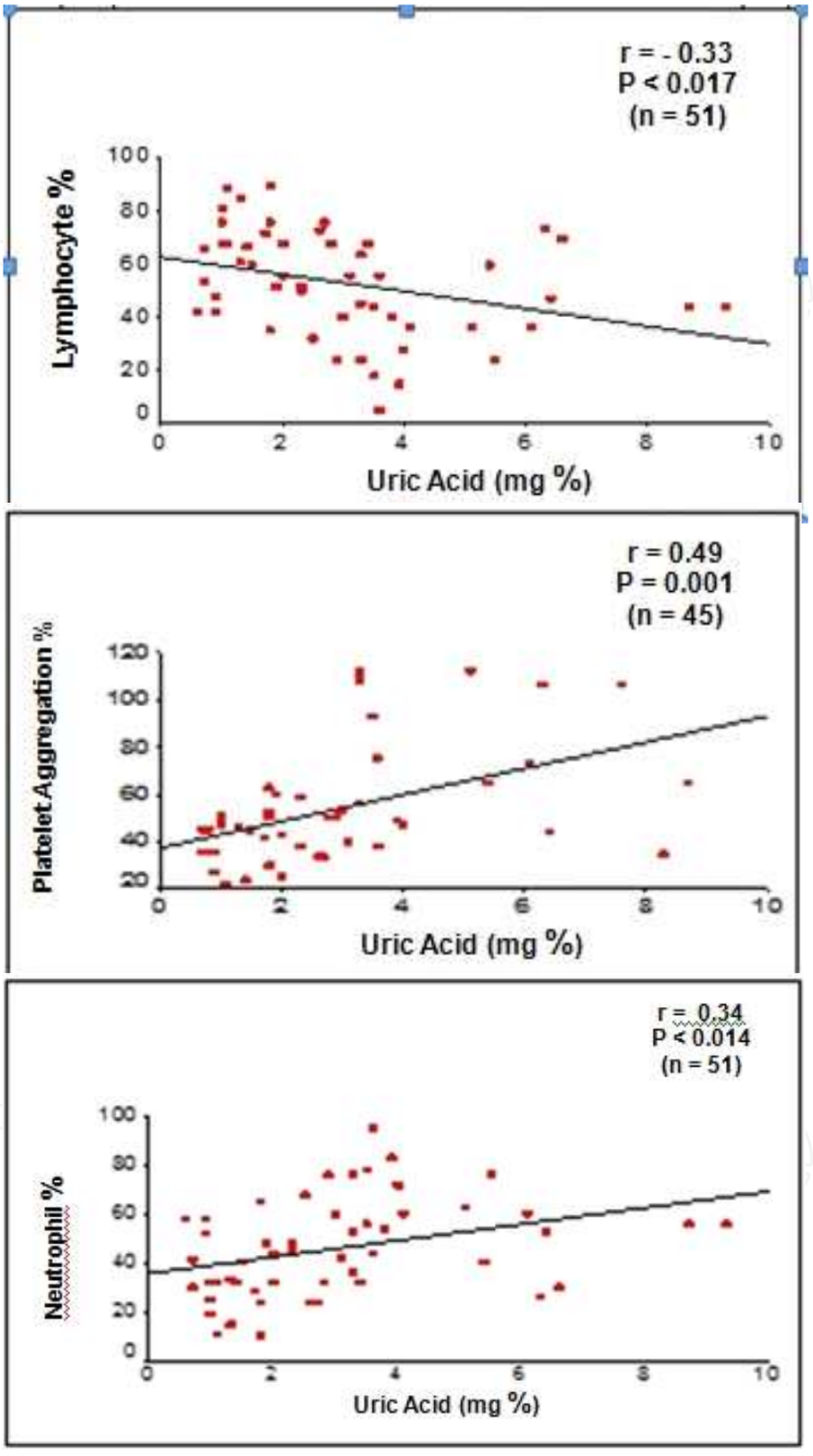

Figure 4. Graphs showing correlations of plasma uric acid versus neutrophil %, lymphocyte%, and platelet aggregation in all the studied groups of rats.

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References

[1] Alderman, M, & Redfern, J. S. (2004). Serum uric acid-a cardiovascular risk factor? Ther Umsch; 61:547.

[2] Baker, J. F, Krishnan, E, Chen, L, & Schumacher, H. R. (2005). Serum uric acid and cardiovascular disease: Recent developments, and where do they leave us? Am J Med; 118:816.

[3] Barhan, D, & Trinder, P. (1972). An improved colour reagent for the determination of blood glucose by the oxidase system. Analyst; 97:142.

[4] Bories, N. P, & Bories, C. (1995). Nitrate determination in biological fluids by an enzymatic one step assay with nitrate reductase. Clin Chem; 41:904.

[5] Desaulniers, P, Marois, S, Popa-nita, O, Gillbert, C, & Naccache, P. H. (2006). Characterization of an activation factor released from human neutrophils after stimulation by triclinic monosodium urate crystals. J Rheumatol;33:928.

[6] Emmerson, B. T. (1996). The management of gout. New England J Med; , 334, 445-51.

[7] Faint, R. W. (1992). Platelet-neutrophil interactions: their significance. Blood Rev; 6:83.

[8] Fam, A. G. (2002). Gout, diet, and the insulin resistance syndrome. J Rheumatol; , 29, 1350-5.

[9] Ganong, W. F. (2005). Energy Balance, Metabolism, and Nutrition. In: Review of Medical Physiology, 22th edition. McGraw-Hill, United State of America., 297-298.

[10] Hediger, M. A, Johnson, R. J, Miyazaki, H, & Endou, H. (2005). Molecular Physiology of urate transport. Physiology; , 20, 125-33.

[11] Hediger, M. A, Johnson, R. J, Miyazaki, H, & Endou, H. (2005). Molecular physiology of urate transport. Physiology; 20:125.

[12] Hoieggen, A, Fossum, E, Reims, H, & Kjeldsen, S. E. (2003). Serum uric acid and hemorheology in borderline hypertensives and in subjects with established hypertension and left ventricular hypertrophy. Blood Press; 12:104.

[13] Kang, D. H, Han, L, Ouyang, X, Kahn, A. M, Kanellis, J, Li, P, Feng, L, Nakagawa, T, Watanabe, S, Hosoyamada, M, Endou, H, Lipkowitz, M, Abramson, R, Mu, W, &

Johnson, R. J. (2005). Uric acid causes vascular smooth muscle cell proliferation by entering cells via a functional urate transporter. Am J Nephrol; 25:425.

[14] Khosla, U. M, Zharikov, S, Finch, J. L, Nakagawa, T, Roncal, C, Mu, W, Krotova, K, Block, E. R, Prabhakar, S, & Johnson, R. J. (2005). Hyperuricemia induces endothelial dysfunction. Kidney Inter; 67:1739.

[15] Mazzali, M, Hughes, J, Kim, Y, Jefferson, J. A, Kang, D, Gordon, K. L, Lan, H. Y, Kivlighn, S, & Johnson, R. J. (2001). Elevated uric acid increases blood pressure in the rat by a novel crystal-independent mechanism. Hypertension; 38:1101.

[16] Mc Lean, L. (2003). The pathogenesis of gout. In: Rheumatology. Hochberg, M.C., Ginsberg, M.H., Kozin, F., O'Malley, M. & McCarty, D.J. (eds.), Mosby, New York., 1903-1918.

[17] Mustard, J. F, & Hegard, T. B. RowSell, H.C. ((1964). Effects of adenosine nucleotides on platelets aggregation and clotting time. J Lab Clin Med; 64:548.

[18] Patterson, R. A, Horsley, E. T, & Leake, D. S. (2003). Prooxidant and antioxidant properties of human serum ultrafiltrates toward LDL: Important role of uric acid. J Lipid Res; 44: 512.

[19] Ruggiero, C, Cherubini, A, Ble, A, Bos, A. J, Maggio, M, Dixit, V. D, Lauretani, F, Bandinelli, S, Senin, U, & Ferrucci, L. (2006). Uric acid and inflammatory markers. Euro Heart J; 27: 1174.

[20] Saito, M, Ishimitsu, T, Minami, J, Ono, H, Ohrui, M, & Matsuoka, H. (2003). Relation of plasma high-sensitivity C-reactive protein to traditional cardiovascular risk factors. Atherosclerosis; 167:73

[21] Serhan, G. C, Lunberg, U, Weissmann, G, & Samuelsson, B. (1984). Formation of leukotriens and hydroxyl acids by human neutrophils and platelets exposed to monosodium urate. Prostaglandins; 4:563.

[22] Singer, J. M, Pratz, C. M, Pader, E, & Elster, S. K. (1957). The latex fixation test: Agglutination test for C-reactive protein and comparison with the capillary precipitin method. Am J Clin Pathol; 28:614.

[23] Waring, W. S, & Esmail, S. (2005). How should serum uric acid concentrations be interpreted in patients with hypertension? Curr Hypertens Rev; 1:89.

[24] Wu, K. K, Papp, A. C, Manner, C. E, & Hall, E. R. (1987). Interaction between lymphocytes and platelets in the synthesis of prostacyclin. J Clin Invest; 6:1601.

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