

CROATIAN INTERNATIONAL PUBLICATIONS

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Ivković V, Jelaković M, Laganović M, Pećin I, Vrdoljak A, Karanović S, Fuček M, Božina T, Kos J, Željковиć Vrkic T, Premužić V, Živko M, Jelaković B. Adiponectin is Not Associated With Blood Pressure in Normotensives and Untreated Hypertensives With Normal Kidney Function. *Medicine (Baltimore)*. 2014;93(28):e250.

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The role of adiponectin in hypertension is still a matter of debate. Obtained conflicting results could be mostly explained with diversity of subjects included in different studies. Our aim was to analyze association of adiponectin with blood pressure (BP) in a group of normotensive and untreated hypertensive subjects. Participants (N=257) were selected from a random sample of 2487 subjects enrolled in an observational cross-sectional study. Subjects with diabetes and chronic kidney diseases were excluded. BP was measured using Omron M6 device following ESH/ESC guidelines. Adiponectin concentration was determined by ELISA. There

were no differences in adiponectin values (mg/L) between hypertensives and normotensives (median 9.75; iqr: 7.44-17.88 vs 11.35; iqr: 7.43-12.63; P=0.17). On univariate linear regression adiponectin was not associated with systolic or diastolic BP (P>0.05). Furthermore, multivariate analysis did not show significant contribution of log-transformed adiponectin either to systolic (β =-0.040; P=0.43) or diastolic BP (β =0.066; P=0.33). In our group of normotensives and untreated hypertensives with normal kidney function adiponectin was not associated with BP even after adjustment for other risk factors. Our results and conclusions should not be extrapolated to subjects with other characteristics.

Lamot L, Borovecki F, Tambic Bukovac L, Vidovic M, Perica M, Gotovac K, Harjacek M. Aberrant expression of shared master-key genes contributes to the immunopathogenesis in patients with juvenile spondyloarthritis. *PLoS One*. 2014;9(12):e115416.

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Association of juvenile spondyloarthritis (jSpA) with the HLA-B27 genotype is well established, but there is little knowledge of other genetic factors with a role in the development of the disease. To date, only a few studies have tried to find those associated genes by obtaining expression profiles, but with inconsistent results due to various patient selection criteria and methodology. The aim of the present study was to identify and confirm gene signatures and novel biomarkers in highly homogeneous cohorts of untreated and treated patients diagnosed with jSpA and other forms of juvenile idiopathic arthritis (JIA) according to ILAR criteria. For the purposes of the research, total RNA was isolated from whole blood of 45 children with jSpA and known HLA genotype, 11 children with oligo- and polyarticular forms of JIA, as well as 12 age and sex matched control participants without diagnosis of inflammatory disease. DNA microarray gene expression was performed in 11 patients with jSpA and in four healthy controls, along with bioinformatical analysis of retrieved data. Carefully selected differentially expressed genes were analyzed by qRT-PCR in all participants of the study. Microarray results and bioinformatical analysis revealed 745 differentially expressed genes involved in various inflammatory processes, while qRT-PCR analysis of selected genes confirmed data universality and specificity of expression profiles in jSpA patients. The present study indicates that jSpA could be a polygenic disease with a possible malfunction in antigen recognition and activation of immunological response, migration of inflammatory cells and regulation of the immune system. Among genes involved in these processes TLR4, NLRP3, CXCR4 and PTPN12 showed almost consistent expression in study patients diagnosed with jSpA. Those genes and their products could therefore potentially be used as novel biomarkers, possibly predictive of disease prognosis and response to therapy, or even as a target for new therapeutic approaches.

Filipović N, Mašek T, Grković I. Expression of nestin in superior cervical ganglia of rats is influenced by gender and gonadectomy. J Chem Neuroanat. 2014;63C:6-12.

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Neurons and glia arise from neural progenitor cells that express nestin. Although substantial changes in neuronal development were observed during the postnatal period, data concerning dynamics of nestin expression in the superior cervical ganglia (SCG) of rat during that period are lacking. It is known that gonadectomy and steroid hormones influence the development of neurons in the SCG during the postnatal period, but there are no data on how they influence the persistence of nestin expression in the SCG cells. The dynamics of nestin expression in the SCG in rats of three age groups, as well as the influence of gender and gonadectomy, was investigated. Three groups of male rats were sacrificed at 2, 3 and 6 months of age. Additional groups of male and female Sprague-Dawley rats were gonadectomized at the age of 2 months. After 30 days, they were sacrificed and SCGs were harvested and processed immunohistochemically. Immunoreactivity for nestin in the SCG was observed in satellite glia, based on their expression of s100. The proportion of neurons that were encircled with nestin-immunoreactive satellite cells (nestin encircled neurons, NEN) decreased between second and third month of age ($p < 0.05$). The proportion of NEN was greater in the NPY+ than in the NPY- subpopulation. The proportion of NEN in the SCG of female rats was significantly higher ($p < 0.05$) than that of both, the male rats and ovariectomized groups. The percentage of these neurons was significantly higher ($p < 0.05$) in orchidectomized, in comparison to male rats. Results show the existence of nestin-immunoreactive satellite cells in the SCG of adult rats. A substantial decrease of nestin expression in SCG cells of rats, after the onset of sexual maturation, was observed. This decrease showed significant sex-dependence and was dramatically influenced by gonadal activity.

Bulum T, Blaslov K, Duvnjak L. Risk factors for development and progression of nonproliferative retinopathy in normoalbuminuric patients with type 1 diabetes. Diabetes Res Clin Pract. 2014;106(3):555-9.

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BACKGROUND: The aim of this study was to evaluate risk factors for development and progression of nonproliferative retinopathy (NPR) in normoalbuminuric patients with type 1 diabetes mellitus (T1DM). MET-

HODS: A total of 223 T1DM with normal renal function and normoalbuminuria were included in this study and followed for 48 months. Photodocumented retinopathy status was made according to the EURODIAB protocol. Urinary albumin excretion rate (UAE) was measured from at least two 24-h urine samples. Possible risk factors for development or progression of NPR were examined in backward stepwise Cox's multiple regression analysis. **RESULTS:** The majority of patients (70%) had no retinopathy while 67 (30%) had NPR at baseline. Patients with NPR were older, had longer duration of diabetes, higher systolic blood pressure, BMI, resting heart rate, UAE and lower estimated glomerular filtration rate ($p \leq 0.04$ for all). After 48 months 24 patients (10.7%) developed NPR or progressed to proliferative retinopathy. Systolic blood pressure (HR 1.03, CI 1.01-1.05, $p=0.02$), UAE (HR 1.14, CI 1.07-1.21, $p<0.001$), and resting heart rate (HR 1.05, CI 1.01-1.09, $p=0.006$) were significantly associated with development or progression of NPR. **CONCLUSIONS:** Our results suggest that retinopathy is present and may progress in T1DM even when coexisting renal disease is excluded. Normoalbuminuric T1DM requires close monitoring for the early detection of retinopathy, especially if they have a higher UAE, systolic blood pressure and resting heart rate.

Karbić VO, Skoda M, Antončić D, Krištofić I, Komar D, Trobonjača Z. Gabapentin-induced changes of plasma cortisol level and immune status in hysterectomized women. *Int Immunopharmacol.* 2014;23(2):530-6.

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AIM: We have examined the effects of gabapentin (GBP) on stress-related changes of cortisol and catecholamines in patients who underwent hysterectomy because of uterine fibrinoids. Additionally, we have observed the effect of GBP on the immune status in the acute stress response to surgery. **METHODS:** Sixty patients scheduled for an abdominal hysterectomy were randomly assigned to the GBP administration 1h before surgery ($n=30$

pts), or to the placebo group ($n=30$ pts). Blood samples were collected before and 24h after the surgery. The intensity of pain was assessed by a visual analogue scale (VAS) every 8h at rest. Immunomodulatory effects of GBP were determined by flow cytometry. We followed the total proportion of CD3(+) lymphocytes, CD3(+)CD4(+), CD3(-)CD8(+), CD19(+) B lymphocytes, CD16(+)CD56(+) CD3(-)NK cells and CD16(+)CD56(+)CD3(+) NKT cells before and 24h after hysterectomy. The plasma cortisol and catecholamines concentration was used to estimate the level of the stress response. **RESULTS:** VAS pain score at rest was significantly lower in the GBP group than in the placebo group ($P=0.003$). Application of GBP significantly decreased the plasma cortisol level 24h after the operation in comparison to the placebo group ($P<0.001$). We found significant positive correlation between the VAS pain score and concentration of cortisol in all patients ($P=0.025$). GBP reduced the concentration of catecholamines ($p<0.05$). The proportion of CD3(+) ($P=0.027$) and CD3(+)CD4(+) cells ($P=0.006$) was significantly lower in the GBP group 24h after operation, while the contribution of CD19(+) ($P=0.033$) was significantly higher. **CONCLUSION:** Preoperative administration of GBP reduced the pain scores at rest in patients at 0, 16 and 24h after abdominal hysterectomy. Additionally, GBP reduced the stress response and changed immune parameters in the reaction to surgery.

Drinovac V, Bach-Rojecky L, Babić A, Lacković Z. Antinociceptive effect of botulinum toxin type A on experimental abdominal pain. *Eur J Pharmacol.* 2014;745:190-5.

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Visceral pain, especially in the abdominal region, represents one of the most common types of pain. Its chronic form is usually very hard to treat by conventional analgesic agents and adjuvants. We investigated the antinociceptive effect of botulinum toxin type A (BTX-A) in male Wistar rats in two models of visceral pain: peritonitis induced by intraperitoneal injection of 1% acetic acid and colitis induced by intracolonic instillation of 0.1% capsaicin. Pain was measured as the number of abdominal wri-

thes. Additionally, referred mechanical sensitivity in the ventral abdominal area was evaluated by von Frey test and the extent of spinal c-Fos expression was immunohistochemically examined. BTX-A significantly reduced the number of abdominal writhes in both models of visceral pain after intrathecal application in a dose of 2U/kg. In the experimental colitis model, BTX-A (2U/kg) reduced both referred mechanical allodynia and c-Fos expression in the dorsal horn of the spinal cord (S2/S3 segments). In contrast to intrathecal administration, BTX-A (2U/kg) administered into the cisterna magna had no effect on pain suggesting that the primary site of its action is a spinal cord.

Koršić M, Kušec V. Serum Leptin and Skeletal Differences between Obese and Non-Obese Patients with Chronic Obstructive Pulmonary Disease. *Obes Facts*. 2014;7(6):399-407.

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OBJECTIVE: Chronic obstructive pulmonary disease (COPD) affects body composition, adipokine secretion, and skeletal integrity. The aim was to determine the association between leptin, body mass (BM) and body composition parameters - fat mass (FM) and fat mass index (FMI), lean tissue mass (LTM), lean tissue mass index (LTMI) and bone mineral density (BMD) in 67 male COPD patients. **METHODS:** BM, body composition and biochemical indicators were measured or calculated using standard methods. Data were analyzed according to groups: non-obese (N = 48, BMI 21.0-29.9 kg/m²) and obese (N = 19, BMI ≥ 30.0 kg/m²). **RESULTS:** In the non-obese group statistically significant correlations were observed: negative ones of age with most BMD T scores, positive ones of BMI with all T scores, FM, FMI, LTMI and leptin, of FMI with leptin and all T scores, and of LTMI with most T scores. In the obese group also statistically significant correlations were found: positive ones of BMI with FMI, LTM, leptin and T scores (trochanter, total hip); of FMI with leptin; and of leptin with total hip T score. **CONCLUSION:** A positive relationship between FMI and BMD was found only in non-obese but not in obese COPD patients. Leptin concentration was associated positively with the total hip T score only in obese COPD patients, suggesting its protective role on the skeleton of obese COPD patients.

Viskovic K, Zidovec-Lepej S, Gorenc L, Grgic I, Lukas D, Zekan S, Dragobratovic A, Begovac J. Cardiovascular markers of inflammation and serum lipid levels in HIV-infected patients with undetectable viraemia. *J Int AIDS Soc*. 2014;17(4 Suppl 3):19548.

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INTRODUCTION: Successfully treated HIV-infected patients may still have an increased risk for cardiovascular morbidity and mortality, which might be related not only to traditional risks, but also to inflammation and dyslipidemia induced by HIV and/or antiretroviral therapy [1, 2]. We examined the relationship of serum lipid levels with plasma biomarkers of inflammation using a composite inflammatory burden score (IBS) from the following seven markers of inflammation: CD40L, tPA, MCP-1, IL-8, IL-6, hCRP and P-selectin. **MATERIALS AND METHODS:** Subjects were selected among consecutive HIV-infected males ≥18 years of age with an undetectable viral load (<50 copies/mL of HIV1-RNA), seen at the University Hospital for Infectious Diseases, Zagreb, Croatia, in the period from January 2012 to March 2013. Plasma inflammatory biomarkers (CD40L, tPA, MCP-1, IL-8, IL-6, hCRP and P-selectin, quantified by bead-based cytometry) >75th percentile were considered elevated and an IBS was constructed as the presence of zero, one, two, or three or more elevated biomarkers. Correlations between the IBS and lipid parameters were examined using Spearman's Rho and by ordered logistic regression proportional odds model to estimate the odds of more elevated (>75th percentile) biomarkers. **RESULTS:** 181 male patients were included into the study, the median age was 46.7 (Q1-Q3, 39.9-55.0) years and the median current CD4 cell count was 553.0 (Q1-Q3, 389-729) per microliter. The patients were mainly treated with two nucleoside reverse transcriptase inhibitor (NRTI) plus one non-NRTI (NNRTI) (N=100, 60.8%) or two NRTI plus lopinavir (N=50, 27.6%). There was a significant correlation between the IBS and serum cholesterol (Rho=0.23, 95% CI, 0.09-0.37), triglycerides (Rho=0.30, 95% CI, 0.16-0.42) and cholesterol/HDL-cholesterol ratio (Rho=0.25, 95% CI 0.11-0.38). In the multivariable model a one unit increase in cholesterol/HDL-cholesterol ratio was associated with a 1.72-fold (95% CI, 1.27-2.33) increased odds of having a greater IBS. One unit

increase (mmol/L) of cholesterol and triglycerides was associated with a 1.41-fold (95% CI, 1.13-1.76) and 1.37-fold (95% CI, 1.18-1.60) increased odds of having a greater IBS, respectively. CONCLUSIONS: Our study suggests that in virologically suppressed patients there is a significant association between markers of inflammation and serum levels of cholesterol and triglycerides as well as the cholesterol/HDL-cholesterol ratio.