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过敏性紫癜血管内皮生长因子含量及其与肾损害指标的相关性*

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摘要 目的:探讨紫癜性肾炎(APN)、过敏性紫癜(AP)和正常对照组血中血管内皮生长因子(VEGF)和肾损害指标的含量变化,以及 APN 中 VEGF 和肾损害指标的相关性,以证实 VEGF 参与了 AP 的部分发病机理。**方法:**采用 ELISA 法检测 APN 组 22 例,AP 组 28 例及健康儿童 30 名为正常对照组的血 VEGF 的含量。生化分析仪检测三组患儿的血清肌酐(Cr), β_2 微球蛋白(β_2 -MG)和双缩脲法检测 24h 尿蛋白定量。比较 APN 组、AP 组和正常对照组之间 VEGF 和肾损害指标的差异,及 APN 组、AP 组分别与肾损害指标血 Cr、 β_2 -MG 和 24h 尿蛋白定量的相关性分析。**结果:**血浆 VEGF 含量 APN 组 > AP 组 > 正常对照组,差异有统计学意义($P < 0.01$),APN 组患儿血 β_2 -MG、Cr 和 24 小时尿蛋白量较 AP 组患儿显著增高($P < 0.01$);AP 组患儿血清 β_2 -MG、Cr 较对照组患儿显著增高,24 小时尿蛋白量无明显差异;APN 组患儿血浆 VEGF 的含量与血 β_2 -MG、血 Cr、24 小时尿蛋白量呈正相关($r > 0$, $P < 0.01$)。**结论:**儿童 AP 血浆 VEGF 含量,APN 比无肾损害的 AP 更高,说明了血浆 VEGF 水平增高与肾损害的程度密切相关,VEGF 参与了 AP 和 APN 血管炎的发生发展过程,并可与肾损害指标反映其病情程度。

关键词:过敏性紫癜;紫癜性肾炎;儿童;血管内皮生长因子

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The Relationship of Plasm Level of Vascular Endothelial Growth Factor and Dysfunction of Kidney in Children Anaphylactoid Purpura*

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ABSTRACT Objective: To explore the relationship between plasm level of vascular endothelial growth factor (VEGF) and children anaphylactoid purpura (AP). **Methods:** The concentrations of VEGF in plasm were detected with enzyme-linked immunosorbent assay in ninety children with AP and thirty healthy children. The serum Cr, β_2 -MG and 24h urine protein quantitative were detected with biochemical analyzer. The concentrations of VEGF serum of AP children Compared with acute phase, remission and healthy control group, and with acute kidney involvement group (APN group) and no renal involvement group (AP group). **Results:** The level of VEGF in acute phase of AP children was significantly higher than those in remission and normal control group; the level of VEGF in remission group was higher than that of normal control group; the level of VEGF in acute phase of APN group was significantly higher than those in AP group and normal control group; The serum concentrations of β_2 -MG and 24h urine protein in APN group was significantly higher than AP group; the serum concentrations of Cr and β_2 -MG in acute phase of AP children was significantly higher than normal control group; the concentrations of serum β_2 -MG, Cr and 24 hour urinary protein were positively correlated with plasma VEGF in APN children in acute phase. **Conclusion:** The concentrations of VEGF in serum increases markedly in acute phase of AP, those in APN is higher than in AP. Increased plasma VEGF level has a close correlation to the degree of AP renal damage, and VEGF is involved in the occurrence and development of AP and APN vasculitis, and can reflect the severity of the disease.

Key words: AP; APN; Children; VEGF

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前言

儿童过敏性紫癜(Anaphylactoid purpura, AP)是儿童最为常见的、反复发作的免疫性疾病,以广泛的毛细血管及小血管

炎为特征。该病累及范围较广,其中尤以肾脏损伤最为严重,有报道其患病率达一半。且有病程长,难以控制的特点^[1-3],严重威胁着儿童的健康成长。目前发病机理尚不清楚,还缺乏特异的早期诊断手段和疗效可靠的治疗。血管内皮生长因子

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(Vascular endothelial growth factor, VEGF)是细胞因子的一种,它在炎症形成中起双重作用,一方面作为保护性反应:VEGF可促进血管内皮细胞的增生,毛细血管形成增多,促进创伤修复的进行。另一方面为炎症反应^[45]:在损伤因子的作用下,血管内皮细胞分泌 VEGF 增多,单核细胞等血细胞具有 Flt-1 受体,它们可在 VEGF 的作用下向血管内皮移动,同时 VEGF 可促内皮释放 von Willebrand 因子及内皮胶原酶,使血管通透性增高,促进单核细胞等聚集并进入组织间隙参与炎症反应。因此检测 VEGF 在儿童 AP 血浆含量是有必要的。我们引入 ELISA 法,检测紫癜性肾炎(anaphylactoid purpura nephritis, APN)、AP 患儿和健康儿童的血浆 VEGF 和肾损害指标含量变化及其 APN 与肾损害指标相关性分析,旨在初步探讨 VEGF 在儿童 AP 的部分发病机理,并发挥特定作用。借此既可拓展专业知识领域,使 VEGF 在 AP 发病中作用更加明确,与肾损害指标的关系得以显现,丰富和充实了 AP 的发病机理;又可拓宽临床科研领域,对临床 AP 的实验室诊断指标寻找新的防治靶点和预后估计提供研究基础。

1 材料和方法

1.1 研究对象及分组

病例选自在内蒙古医科大学附属医院儿科病房收治的 AP 初诊患儿 50 例,其中 28 例无肾损害为 AP 组,男 15 例,女 13 例,年龄最小 3 岁,最大 13 岁,平均年龄(7.58±2.48)岁;22 例伴有肾损害为 APN 组,男 9 例,女 13 例,年龄最小 4 岁,最大 12 岁,平均年龄(7.22±3.24)岁;于同时间、同群体中选择健康体检儿童 30 名为正常对照组,男 16 名,女 14 名,年龄最小 2 岁,最大 14 岁,平均年龄(8.04±6.23)岁。三组间年龄、性别构成比较差异均无统计学意义(均 $P>0.05$)。

1.2 诊断、纳入和排除标准

AP 的诊断及肾脏等各器官受损标准见第七版《诸福棠实用儿科学》有关章节^[6]。所谓 AP 组系指只要无肾损害,不管有无关节等其他器官损害,均列入 AP 组;所谓 APN 组系指只要有肾损害,不管有无其他器官损害,均列入 APN 组。AP 和 APN 组只要符合诊断标准则纳入研究。所有研究对象排除其他血管炎和免疫性疾病史和家族史,对照组近 4 周内无感染史。

1.3 标本收集

研究对象于早晨用 EDTA 抗凝管抽取静脉血 3 mL,4℃ 保存,并尽快以 1500 转/分离心 20 分,将血浆于 -20℃ 冰箱保存备检。同时生化分析仪检测三组患儿的血清肌酐(Cr), β_2 微球蛋白(β_2 -MG)和双缩脲法检测 24h 尿蛋白定量。

1.4 VEGF 的检测

血浆复温后,按照酶联免疫反应试剂盒操作说明书操作,最后用酶标仪 5 分钟内阅读 OD450 值,与试剂盒标准曲线对比,计算血管内皮生长因子的血浆浓度。

1.5 统计学处理

应用美国 SPSS12.0 版本统计软件做统计分析。计量资料描述以均数±标准差表示。年龄和性别构成比较分别用单因素方差分析和行×列 χ^2 检验。三组间 VEGF 浓度及肾损害指标的均数比较在方差齐同的条件下,应用单因素方差分析,并做两两比较,相关分析采用 Pearson 相关分析。

2 结果

2.1 血浆 VEGF 含量变化

APN 组、AP 组和正常对照组血浆 VEGF 含量,比较差异有统计学意义($P<0.01$)见表 1。

表 1 APN 组、AP 组和正常对照组血浆 VEGF 含量比较

Table 1 The VEGF of APN,AP and normal group

分组 Group	例数 Cases	VEGF 含量($\mu\text{g/L}$) VEGF
APN 组 APN group	22	244.56±51.12 ^{①②}
AP 组 AP group	28	155.71±50.48 ^③
正常对照组 Normal group	30	54.91±40.07
F		95.789
P		$P=0.000<0.01$

注:① APN 组与 AP 组比较, $P=0.000<0.01$; The comparment of APN and AP, $P=0.000<0.01$.

② APN 组与正常对照组比较, $P=0.000<0.01$; The comparment of APN and NP, $P=0.000<0.01$.

③ AP 组与正常对照组比较, $P=0.000<0.01$,The comparment of AP and NP, $P=0.000<0.01$.

2.2 血 β_2 -MG、血 Cr、24 小时尿蛋白含量变化

APN 组、AP 组和正常对照组血 β_2 -MG、Cr 含量,经比较差异有统计学意义, $P<0.01$,见表 2。

APN 组和 AP 组 24 小时尿蛋白含量,经比较差异有统计学意义, $P<0.01$,见表 2。AP 组和正常对照组 24 小时尿蛋白含量,经比较差异无统计学意义, $P>0.05$,见表 2。

2.3 APN 患儿血 VEGF、血 β_2 -MG、血 Cr 和 24 小时尿蛋白量的相关分析

APN 患儿血 VEGF 含量分别与血 β_2 -MG、血 Cr 和 24 小时尿蛋白量比较,呈显著正相关, $P<0.01$,见表 3。

3 讨论

AP 在儿童血管性疾病中较为常见,常涉及多脏器多系统损害的疾病。其临床特点除皮肤紫癜外,常有关节肿痛、剧烈腹痛、便血和肾脏受损等,尤其是 APN,其病程有时迁延不愈,目前发病机制主要是由机体免疫调节功能障碍导致微血管内皮

表 2 APN 组、AP 组和正常对照组血 β 2-MG、血 Cr 和 24 小时尿蛋白含量比较Table 2 The β 2-MG, Cr and urinary protein of APN, AP and NP

分组 Group	例数 Cases	血 β 2-MG(mg/l) β 2-MG	血 Cr(umol/L) Cr	24 小时尿蛋白(g/L) urinary protein
APN 组 APN group	22	5.02 $\pm$ 0.98 ^{①②}	318.67 $\pm$ 112.57 ^{①②}	2.27 $\pm$ 1.57 ^{①②}
AP 组 AP group	28	3.71 $\pm$ 0.79 ^③	143.69 $\pm$ 89.72 ^③	0.79 $\pm$ 0.28
正常对照组 Normal group	30	1.95 $\pm$ 0.87	89.94 $\pm$ 35.51	0.56 $\pm$ 0.22
F		94.257	96.674	82.673
P		P=0.000<0.01	P=0.000<0.01	P=0.000<0.01

注:① APN 组与 AP 组比较, P=0.000<0.01; The comparment of APN and AP, P=0.000<0.01.

② APN 组与正常对照组比较, P=0.000<0.01; The comparment of APN and NP, P=0.000<0.01.

③ AP 组与正常对照组比较, P=0.000<0.01, The comparment of AP and NP, P=0.000<0.01.

表 3 APN 组 VEGF 分别与血 β 2-MG、血 Cr 和 24 小时尿蛋白量相关分析结果Table 3 The relationship of VEGF with β 2-MG, Cr and urinary protein on APN

VEGF 与血 β 2-MG VEGF and β 2-MG		VEGF 与血 Cr VEGF and Cr		VEGF 与 24 小时尿蛋白量 VEGF and 24 hours urinary protein	
r	P	r	P	r	P
0.785	P=0.000<0.01	0.482	P=0.000<0.01	0.887	P=0.000<0.01

细胞免疫性损害,使肾脏局部区域发生缺血性改变,从而损伤肾脏^[7-15]。

VEGF 多在正常组织和血浆中低水平表达,有许多学者研究发现,VEGF 过表达可损伤内脏器官微血管尤其是肾脏小血管和基底膜的通透性^[16-19]。已有文章报道^[20],VEGF 与某些自身免疫性血管炎症性疾病(如川崎病、韦格纳肉芽肿、类风湿性关节炎等)密切相关。Topaliglu 等通过对 22 例 AP 患儿血浆 VEGF 及皮损处皮肤的免疫组化检测发现,AP 急性期血浆 VEGF 浓度较缓解期及健康对照组有显著性增高,认为 AP 中的血管内皮损伤是一系列炎症反应和免疫过程的关键因素,由于 VEGF 可以促进血管内皮细胞功能改变,提示 VEGF 可能在 AP 的发生、发展过程中,参与了复杂而协调的炎症过程,并与 AP 的疾病进展相关,作用于疾病的不同时期。因此探讨 VEGF 在 AP 病程中血 VEGF 含量变化及与肾损害指标的相关性是有很大意义的,其有望提示 AP 可能的部分发病机理。但有关研究 VEGF 在 AP 病程中含量变化国内外报道较少,经检索查新,研究 VEGF 在 APN 中与肾损害指标相关性分析只有 2 篇^[17-24]。本研究发现:血 VEGF 含量在 APN 组均高于 AP 组和对照组,AP 组亦高于对照组,差异有统计学意义(P<0.01,其 95% 可信区间内均不包含 1)。APN 患儿血 VEGF 含量与血 β 2-MG、血 Cr、24 小时尿蛋白量呈显著正相关(P<0.01)。

本次研究结果与以往研究结果相似,也验证了其真实性,也说明了 VEGF 参与了 APN 血管炎的发生发展过程,并与 AP 的疾病进展相关。VEGF 含量的增高与肾脏损害的程度密切相关,并可反映其病情程度。因此,检测血浆 VEGF 含量有可能成为一个 AP 变成 APN 的早期预后诊断指标。同时通过 VEGF 的单克隆抗体,可阻断炎症因子的级联反应,有望为 AP 和 APN 的免疫治疗增添新的途径,有良好的临床应用前景。

参考文献(References)

[1] Zeng HS, Xiong XY, Chen YY, et al. Gene polymorphism of vascular

endothelial growth factor in children with Henoch-Schonlein purpura nephritis[J]. Chinese Modern Pediatr, 2009, 11(6): 417-421

[2] McCarthy HJ, Tizard EJ. Clinical practice:Diagnosis and management of Henoch-schonlein purpura[J]. Eur J Pediatr, 2010, 169(6): 643-650

[3] Peng S, He X, Yi Z, et al. Serum and urine VEGF concentration of different pathological types in children with Henoch Schonlein purpura nephritis[J]. The Journal of Central Southern University, 2009, 34(12): 1209-1215

[4] Zeng XQ, Xu ZC, Dang XQ, et al. Expression of vascular endothelial growth factor is related to microvessel injury of renal interstitium in children with Henoch Schönlein purpura nephritis[J]. ZChinese Modern Pediatr, 2009, 11(9): 717-721

[5] Zeng HS, Xiong XY, Chen YY, et al. Gene polymorphism of vascular endothelial growth factor in children with Henoch-Schonlein purpura nephritis[J]. Chinese Modern Pediatr, 2009, 11(6): 417-421

[6] 胡亚美,江载芳,主编. 诸福棠实用儿科学[M]. 第 7 版. 北京:人民卫生出版社,2002: 688-690

Hu Ya-mei, Jiang Zai-fang, editor. Zhu FT Pediatr [M]. 7th editiom, Beijing: People's medical publishing house, 2002: 688-690

[7] Rajewska J, Gawrych E, Fischer K, et al. Estimation of vascular endothelial growth factor and placental growth factor serum levels' in infant with hemangioma and population of healthy infants[J]. Ann Acad Med Stetin, 2012, 58(2): 5-10

[8] Kalmeh ZA, Azarpira N, Mosallaei M, et al. Genetic polymorphisms of vascular endothelial growth factor and risk for retinopathy of prematurity in South of Iran[J]. Mol Biol Rep, 2013, 40(7): 4613-4618

[9] Bain JM, Moore L, Ren Z, et al. Vascular endothelial growth factors A and C are induced in the SVZ following neonatal hypoxia-ischemia and exert different effects on neonatal glial progenitors [J]. Transl Stroke Res, 2013, 4(2): 158-170

[10] Aho OM, Lehenkari P, Ristiniemi J, et al. The mechanism of action of induced membranes in bone repair [J]. J Bone Joint Surg Am,

- 2013, 95(7): 597-604
- [11] Forlenza GP, Stewart MW. Diabetic retinopathy in children[J]. *Pediatr Endocrinol Rev*, 2012, 10(2): 217-226
- [12] Zhao M, Yin Y, Guo F, et al. Placental expression of VEGF is increased in pregnancies with hydatidiform mole: Possible association with developing very early onset preeclampsia[J]. *Early Hum Dev*, 2013, 89(8): 583-588
- [13] Gnanaguru G, Brunken WJ. The cell-matrix interface: a possible target for treating retinal vascular related pathologies[J]. *J Ophthalmic Vis Res*, 2012, 7(4): 316-327
- [14] Tateossian H, Morse S, Parker A, et al. Otitis media in the Tgfr knockout mouse implicates TGF β signalling in chronic middle ear inflammatory disease[J]. *Hum Mol Genet*, 2013, 22(13): 2553-2565
- [15] Joshi MS, Berger PJ, Kaye DM, et al. Functional relevance of genetic variations of endothelial nitric oxide synthase and vascular endothelial growth factor in diabetic coronary microvessel dysfunction[J]. *Clin Exp Pharmacol Physiol*, 2013, 40(4): 253-261
- [16] Gordon K, Schulte D, Brice G, et al. Mutation in vascular endothelial growth factor-C, a ligand for vascular endothelial growth factor receptor-3, is associated with autosomal dominant milroy-like primary lymphedema[J]. *Circ Res*, 2013, 112(6): 956-960
- [17] Shroff RC, Price KL, Kolatsi-Joannou M, et al. Circulating angiopoietin-2 is a marker for early cardiovascular disease in children on chronic dialysis[J]. *PLoS One*, 2013, 8(2): e56273
- [18] Labied S, Delforge Y, Munaut C, et al. Isoform 111 of vascular endothelial growth factor (VEGF111) improves angiogenesis of ovarian tissue xenotransplantation[J]. *Transplantation*, 2013, 95(3): 426-433
- [19] Cuadrado E, Jansen MH, Anink J, et al. Chronic exposure of astrocytes to interferon- α reveals molecular changes related to Aicardi-Goutieres syndrome[J]. *Brain*, 2013, 136(Pt 1): 245-258
- [20] Mita-Mendoza NK, van de Hoef DL, Lopera-Mesa TM, et al. A potential role for plasma uric acid in the endothelial pathology of *Plasmodium falciparum* malaria[J]. *PLoS One*, 2013, 8(1): e54481
- [21] 赵讯, 王学敏, 林海英. 2型糖尿病肾病地区发病率及危险因素分析[J]. *现代仪器与医疗*, 2013, 19(1): 54-56
- Zhao Xun, Wang Xue-min, Lin Hai-ying. Analysis of incidence and risk factors of type 2 diabetic nephropathy [J]. *Morden instruments and medical treatment*, 2013, 19(1): 54-56
- [22] 曾德理, 曾惠琼, 周文娟. 酸放散、热放散联合检测新生儿 ABO 溶血病结果分析[J]. *现代仪器与医疗*, 2012, 17(3): 56-58
- Zeng De-li, Zeng Hui-qiong, Zhou Wen-juan. The analysis results of ABO hemolytic disease of the newborn combined detection of acid elution[J]. *Morden instrument and medicine*, 2012, 17(3): 56-58
- [23] 杨蕊. 合理降低螺旋 CT 在儿童扫描中的剂量探讨 [J]. *现代仪器*, 2011, 16(7): 30-31
- Yang Rui. Reasonable to reduce dose spiral CT scanning in children [J]. *Morden instrument*, 2011, 16(7): 30-31
- [24] 李进. 轻度胃肠炎伴婴幼儿良性惊厥临床观察及随访分析 [J]. *现代仪器与医疗* 2013, 18(2): 235-237
- Li Jin. Analysis of clinical observation and follow-up of benign infantile convulsions associated with mild gastroenteritis [J]. *Morden instrument and medicine*, 2013, 18(2): 235-237

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- Infectious Diseases and Phraseology, Hepatology branch of Chinese Medical Association. Viral hepatitis prevention and treatment programs[J]. *Chinese Journal of Hepatology*, 2000, 8(6): 324-329
- [9] Povero D, Busletta C, Novo E, et al. Liver fibrosis: a dynamic and potentially reversible process[J]. *HistolHistopathol*, 2010, 25(8): 1075-1091
- [10] Pinzani M, Macias-Barragan J. Update on the pathophysiology of liver fibrosis[J]. *Expert Rev GastroenterolHepatol*, 2010, 4(4): 459-472
- [11] Jarcuska P, Janicko M, Veseliny E, et al. Circulating markers of liver fibrosis progression[J]. *ClinChimActa*, 2010, 411(15-16): 1009-1017
- [12] Martinez S M, Crespo G, Navasa M, et al. Noninvasive assessment of liver fibrosis[J]. *Hepatology*, 2011, 53(1): 325-335
- [13] Adams L A. Biomarkers of liver fibrosis [J]. *J GastroenterolHepatol*, 2011, 26(5): 802-809
- [14] Castera L, Pinzani M. Biopsy and non-invasive methods for the diagnosis of liver fibrosis: does it take two to tango [J]. *Gut*, 2010, 59(7): 861-866
- [15] Germani G, Hytioglou P, Fotiadu A, et al. Assessment of fibrosis and cirrhosis in liver biopsies: an update [J]. *Semin Liver Dis*, 2011, 31(1): 82-90
- [16] Verveer C, Zondervan P E, Ten K F, et al. Evaluation of transient elastography for fibrosis assessment compared with large biopsies in chronic hepatitis B and C[J]. *Liver Int*, 2012, 32(4): 622-628
- [17] Scott D R, Levy M T. Liver transient elastography (Fibroscan): a place in the management algorithms of chronic viral hepatitis [J]. *AntivirTher*, 2010, 15(1): 1-11
- [18] Barrault C, Roudot-Thoraval F, Tran V N J, et al. Non-invasive assessment of liver graft fibrosis by transient elastography after liver transplantation[J]. *Clin Res HepatolGastroenterol*, 2013, 37(4): 347-352
- [19] Carey E, Carey W D. Noninvasive tests for liver disease, fibrosis, and cirrhosis: Is liver biopsy obsolete [J]. *Cleve Clin J Med*, 2010, 77(8): 519-527
- [20] Cohen E B, Afdhal N H. Ultrasound-based hepatic elastography: origins, limitations, and applications[J]. *J ClinGastroenterol*, 2010, 44(9): 637-645