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外周血单个核细胞计数与 2 型糖尿病的相关性分析 *

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摘要 目的:将传统的外周血单个核细胞分离计数方法加以改进,并探讨 2 型糖尿病(DM)患者外周血单个核细胞数量与疾病的相关性。**方法:**密度梯度离心分离 30 例健康体检者单个核细胞,不同染色稀释液重悬细胞,充入改良牛鲍计数板连续计数 5 天,计算批内、批间变异;采用上述方法分离 2 型 DM 患者抗凝全血 29 例,计算单个核细胞数量;采用全自动生化分析仪检测对照组和 2 型 DM 组患者血脂。**结果:**两组的单个核细胞经 3 种前处理染液稀释重悬细胞后,计数结果间无统计学差异($P > 0.05$);2 型 DM 组与健康对照组比较,外周血单个核细胞计数绝对值无显著性差异($P > 0.05$),血清 TG、TC、LDL 水平明显升高,差别具有统计学意义($P < 0.05$),而其他指标结果间比较无差异。在对照组中,单个核细胞计数结果与白细胞呈正相关($r = 0.611, P < 0.05$),与血清 TC、LDL 水平呈负相关($r_1 = -0.550, r_2 = -0.605, P < 0.05$)。而 T2DM 组,单个核细胞计数结果仅与 LDL 水平呈负相关($r = -0.104, P < 0.05$)。**结论:**外周血单个核细胞数与 2 型糖尿病无明显相关性。

关键词: 单个核细胞计数; 2 型糖尿病

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Correlation Analysis between Peripheral Blood Mononuclear Cell Numbers and Type 2 Diabetes Mellitus*

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ABSTRACT Objective: To improve traditional counting method of peripheral blood mononuclear cells, and investigate association between quantity of peripheral blood mononuclear cell and type 2 diabetes mellitus (DM). **Methods:** Mononuclear cells of 30 healthy people were obtained by density gradient centrifugation, mixing with different colored dilution and counting in continuous 5 days, establishing interassay variation and intraassay coefficient of variation; Peripheral blood mononuclear cell of 29 type 2 diabetic patients were gained by above-mentioned method; Plasma lipids were measured by 7600-020E automatic biochemistry analyzer. **Results:** Mononuclear cells of healthy people were mixed by staining solutions, there is no statistics significance among counted numbers ($P > 0.05$); Comparing with healthy control, there is no statistics significance between absolute value of Mononuclear cells of type 2 diabetic patients ($P > 0.05$), serum TG, TC, LDL were higher obviously, with statistics significance ($P < 0.05$), there is difference among results of other index. In control, counted numbers of mononuclear cells were positive correlation with leucocyte ($r = 0.611, P < 0.05$), negative correlation with TC, LDL ($r_1 = -0.550, r_2 = -0.605, P < 0.05$). In type 2 diabetic group, counted numbers of mononuclear cells was only negative correlation with LDL ($r = -0.104, P < 0.05$). **Conclusion:** Numbers of mononuclear cells is no obviously association with type 2 diabetic patients.

Key words: Numbers of mononuclear cells; Type 2 diabetic mellitus

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前言

糖尿病是由多种病因引起的以慢性高血糖为特征的代谢紊乱综合征,慢性血管病变是 2 型糖尿病(T2DM)常见病理损伤。近年来认为,血管内皮祖细胞(EPCs)是一类具有高增殖潜能的前体细胞,其在机体自身调节、维持血管内环境稳定方面起到重要作用^[1]。研究显示,受损血管的修复反映损害正在进

行,血管受损程度和修复能力的不平衡是导致动脉粥样硬化形成的原因,可能与 EPCs 数量的降低直接影响内皮组织修复能力有关^[2]。大量研究结果表明 EPCs 具有修复内皮功能,而从外周血分离单个核细胞(PBMC),经诱导培养获得 EPCs,加磁粒子标记后自体输注治疗 T2DM 血管损伤的动物模型已为当今的研究热点^[3,4]。亦有研究表明 PBMC 在功能上与 T2DM 发生发展有密切联系^[5]。本实验将传统的外周血单个核细胞分离计

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数方法加以改良,加入不同浓度染色液以提高单个核细胞计数准确率,并探讨2型糖尿病患者外周血单个核细胞数量与动脉硬化程度的相关性。

1 材料与方法

1.1 实验材料

人淋巴细胞分离液(上海华精生物高科技有限公司),稀释液A、B、C(配方:A:1×PBS;B:1%冰醋酸;C:1%冰醋酸及0.5%龙胆紫),1×磷酸盐缓冲液(PBS)为本实验室配制,TC、HDL和LDL检测试剂盒购自日本协和医药株式会社;TG检测试剂盒购自中生北控生物科技股份有限公司。

1.2 实验对象

T2DM组:2012年3月到5月南京市鼓楼医院内分泌科收治的T2DM患者29例,男15例,女14例,年龄(58.7 ± 15.6)岁,均符合WHO的糖尿病诊断标准。健康对照组:门诊体检健康者30例,男女各15例,年龄(57.7 ± 13.8)岁,均排除心脑血管

病变、肾脏病、感染、手术及糖尿病等。

1.3 实验方法

1.3.1 外周血单个核细胞分离计数 (1)体检正常人的EDTA抗凝全血10例,与1×PBS 1:1混匀;吸1mL稀释全血缓缓加入置有1mL Ficoll 分离液上层,离心2000 rpm,20 min;吸取白膜层至清洁15mLEP管中,PBS清洗两遍,离心1500rpm,10min;弃上清后,重悬至1mL;吸25 μl 细胞悬液分别与稀释液A、B、C 25 μL混匀,静止3 min,充池计数;重复计数5天并记录。

$$\text{外周血单个核细胞数} = \frac{X_1 + X_2 + X_3 + X_4}{4} \times 10 \times 4 \times 10^6 \text{ 个/L}$$

(2)2型糖尿病患者EDTA抗凝全血采用稀释液处理细胞进行细胞计数,方法同上。

1.3.2 血脂指标检测 抽取两组对象肘静脉血2mL,室温静置30 min,及时分离血清,检测TC、TG、HDL和LDL。TC测定采用胆固醇氧化酶法、TG测定采用GPO-PAP法、HDL测定采用化学修饰酶法、LDL测定采用选择性可溶化法,检测仪器为

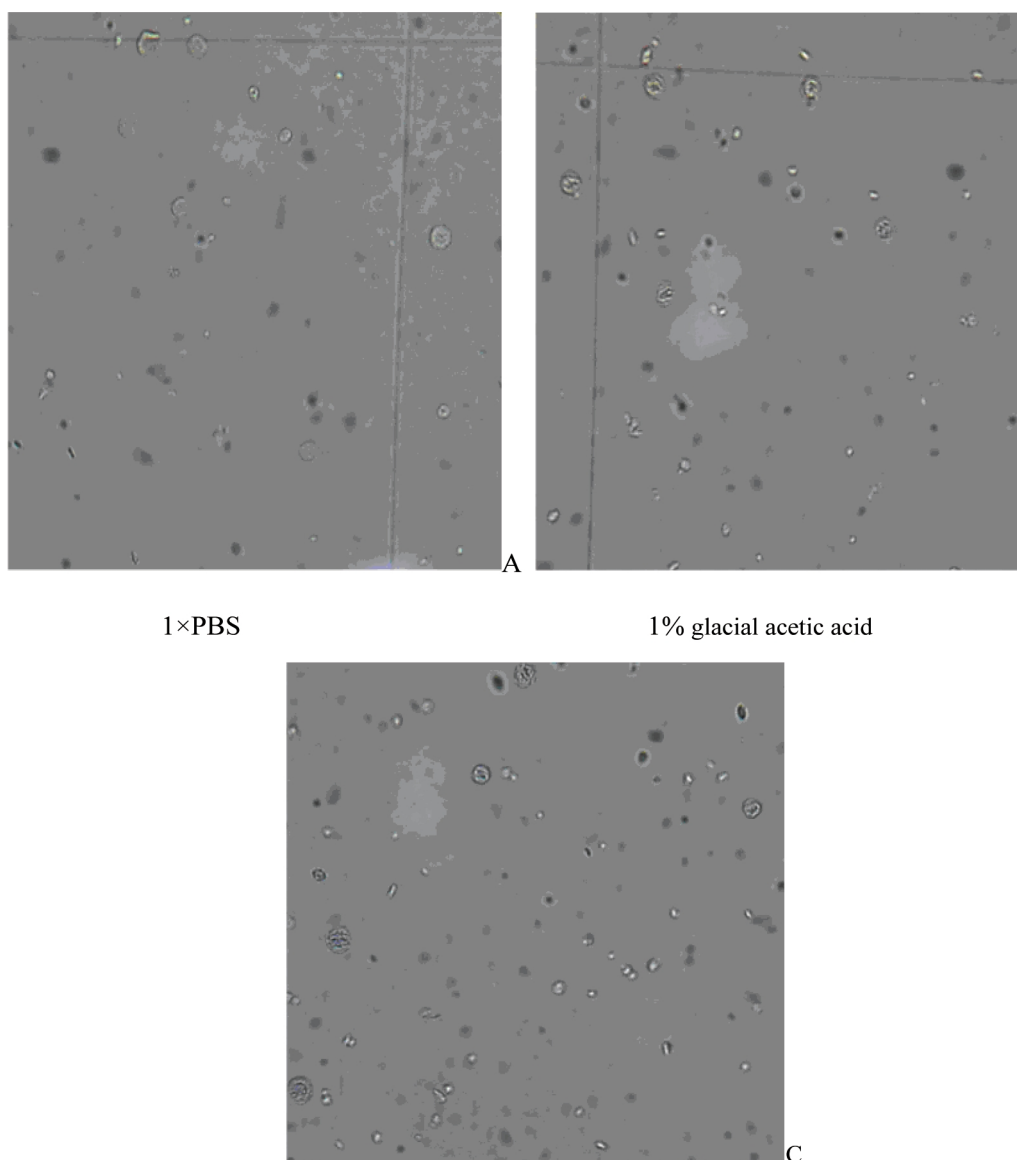


图1

Fig. 1 Graph1 cytes handled by 3 dilutions in test under microscope($\times 40$)

*注:A,B,C 分别代表 1×磷酸缓冲液、1%冰醋酸、1%冰醋酸加 0.5%龙胆紫。

*Note: A, B, C represents 1×PBS, 1% glacial acetic acid, 1% glacial acetic acid+0.5%Methylrosanilinium Chloride.

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1.4 统计学分析

用 SPSS13.0 软件进行统计分析, 计量资料用 $\bar{x} \pm s$ 表示, 组间差异采用 q 检验, 单个核细胞数量与各生化指标之间的关系采用双变量相关分析, 以 $P < 0.05$ 为具有统计学意义。

2 结果

2.1 不同稀释液处理细胞计数

表 1 健康对照组 3 种稀释液处理细胞计数统计($\bar{x} \pm s$)

Table1 Results of cell counting handled by 3 dilutions in healthy control($\bar{x} \pm s$)

	Mononuclear cell(10 ⁹ /L)	Intra CV(%)	Inter CV(%)	P
Dilution A	1.65± 0.61	2.70	4.18	> 0.05
Dilution B	1.62± 0.56	2.89	5.05	> 0.05
Dilution C	1.67± 0.57	2.93	4.60	> 0.05

* 注:A,B,C 分别代表 1× 磷酸缓冲液、1%冰醋酸、1%冰醋酸加 0.5%龙胆紫。

Note : A:PBS,B:1% glacial acetic acid,C:1% glacial acetic acid+0.5% gentian viole .

表 2 糖尿病组和健康对照组单个核细胞及一般资料的比较($\bar{x} \pm s$)

Table 2 Comparison of peripheral blood mononuclear cells and general information in T2DM and control group($\bar{x} \pm s$)

	T2DM (n=29)	Control (n=30)	P
Age(岁)	58.7± 15.6	57.7± 13.8	> 0.05
Mononuclear cell(10 ⁹ /L)	1.57± 0.64	1.62± 0.56	> 0.05
White blood cell(10 ⁹ /L)	7.38± 2.14	6.78± 1.56	> 0.05
Lymphocyte+monocytes(10 ⁹ /L)	2.37± 0.98	2.38± 0.55	> 0.05
TG (mmol/L)	2.11± 1.59	1.60± 0.98	< 0.05
TC (mmol/L)	4.30± 1.50	5.03± 0.94	< 0.05
HDL (mmol/L)	1.09± 0.45	1.33± 0.40	> 0.05
LDL (mmol/L)	2.34± 1.04	2.84± 0.77	< 0.05

2.3 外周血单个核细胞与血脂指标相关性分析

在对照组中, 单个核细胞计数结果与白细胞呈正相关($r=0.611$, $P < 0.05$), 与血清 TC、LDL 水平呈负相关($r_1=-0.550$, $r_2=-0.605$, $P < 0.05$)。而 T2DM 组, 单个核细胞计数结果仅与 LDL 水平呈负相关($r=-0.104$, $P < 0.05$), 与其它指标间无明显相关性。

3 讨论

本实验, 外周血经密度梯度离心分离所得单个核细胞, 用 1%冰醋酸处理后可破坏红细胞并使细胞核明显, 有助于细胞辨别。1%冰醋酸加 0.5%龙胆紫处理组单个核细胞核不仅形态突出且核被染成蓝色, 提高了计数速度但颜色会在一定程度上干扰核的观察。实验中湿染液除龙胆紫外还曾用瑞吉及亚甲蓝两种染液, 三者相比之下龙胆紫具有用量少、着色快、深的优点。本法不足为重悬的细胞不能保存较长时间, 4℃ 保存 5 天后部分单个核细胞出现胀大坏死、核破裂, 不能计数。实验取材为 EDTA 抗凝全血, 不同于多数文献中采用的肝素抗凝全血。肝素不影响细胞形态但能引起白细胞聚集, 可经过分离、洗涤和混匀过程恢复成单个细胞而不影响细胞计数。EDTA 对红白细

与 PBS 稀释的细胞相比, 1%冰醋酸处理组细胞核较明显且核形态较易观察。0.5%龙胆紫可使细胞核染成明显的蓝色(图 1)。30 例样本 3 种稀释液处理细胞计数结果见表 1。

2.2 T2DM 组与对照组一般资料及血脂指标比较

与对照组比较, T2DM 组 TG、TC 及 LDL 水平明显升高, 差别具有统计学意义($P < 0.05$), 而其他指标结果间比较无差异。见表 2。

胞形态影响很小, 但当血液浓度大于 1.5g/L 时对淋巴、单核细胞形态有影响^[6], 与对照组比较, T2DM 组 TG、TC、LDL 水平明显升高, 差别具有统计学意义($P < 0.05$), 而其他指标结果间比较无差异。相关性分析研究中, 对照组单个核细胞计数结果与白细胞呈正相关($r=0.611$, $P < 0.05$), 而与血清 TC、LDL 水平呈负相关($r_1=-0.550$, $r_2=-0.605$, $P < 0.05$)。T2DM 组, 单个核细胞计数结果仅与 LDL 水平呈负相关($r=-0.104$, $P < 0.05$)。T2DM 患者常伴脂代谢紊乱, 葡萄糖利用障碍, 机体为满足从脂类获取能量的需求, 外周血单个核细胞过氧化物酶体脂肪酸 β 氧化途径显著增加, 过氧化物酶体脂肪酸 β 氧化的活性随血清 TG、LDL-C 水平的增加而增加, 表明 DM 状态下过氧化物酶体脂肪酸 β 氧化活性的升高与异常升高的血脂有关^[10-14]。EPCs 培养时间需一周以上, 复杂且成本高。若外周血单个核细胞绝对值与 2 型糖尿病发生发展有相关性, 则可以辅助判断疾病发生的可能性, 为后面做 EPCs 培养做初筛。本实验结果证实两者之间并不存在显著相关性, 可能因素为: (1)EPCs 占单个核细胞比例小。正常人外周血 EPCs 数量极少, 从人外周血单个核细胞中分离出来的 EPCs 仅为 0.05%-0.1%^[15-20], 当有血管损伤时, 在各种因素刺激下释放增加, 但数量相对于单个核细胞中淋巴及单核

细胞而言仍然较小,不足以使单个核细胞总数发生明显改变,达不到统计学意义;(2)并发症因素。不论1型还是2型糖尿病都会引发复杂的并发症,炎症的发生可伴有单个核细胞激活增加或抑制浸润而减少,本文样本为2型糖尿病总体内随机采样,可能因不同病理状态下细胞本身的应激反应不同而不能反映某种单一变化趋势。

本研究中外周血单个核细胞绝对值计数与2型糖尿病之间无明显相关性,但与患病不伴并发症或伴有某一特定并发症的患者仍不能排除存在相关可能,需继续收集2型糖尿病并发血管硬化的典型病例来进一步分析外周血单个核细胞数目及功能与疾病的相关性,期待能够成为2型糖尿病动脉硬化早期监测的常规检测指标。

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