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血清 ghrelin、 β -EP、BMEC 在手足口病中的水平变化及诊断价值*

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摘要 目的:探讨血清生长激素释放肽(ghrelin)、 β -内啡肽(β -EP)、脑微血管内皮细胞(BMEC)在手足口病中的水平变化及诊断价值。**方法:**选择2017年6月至2019年10月于我院进行治疗的150例手足口病患者进行研究,设为研究组,并选择我院120例儿童保健科查体患儿作为对照组,分析血清 ghrelin、 β -EP、BMEC 水平变化情况及其预测价值。**结果:**研究组血清 ghrelin 水平显著低于对照组, β -EP、BMEC 水平显著高于对照组,差异显著($P<0.05$);II 期患儿血清 ghrelin 显著高于 III 期、IV 期患者,血清 β -EP、BMEC 显著低于 III 期、IV 期患者,III 期血清 ghrelin 显著高于 IV 期患者,血清 β -EP、BMEC 显著低于 IV 期患者,差异显著($P<0.05$);ROC 结果显示,血清 ghrelin 预测手足口病的 AUC 为 0.955,灵敏度为 85.62%,特异度为 87.45%,截断值为 12.91 ng/mL;血清 β -EP 预测手足口病的 AUC 为 0.801,灵敏度 82.25%,特异度为 83.56%,截断值为 196.87 ng/L;血清 BMEC 预测手足口病的 AUC 为 0.974,灵敏度为 84.18%,特异度为 85.97%,截断值为 56.28 个/mL,联合检测较单独检测灵敏度、特异度更高($P<0.05$)。**结论:**血清 ghrelin、 β -EP、BMEC 与手足口病患者病情有关,有利于指导临床诊疗。

关键词:生长激素释放肽; β -内啡肽;脑微血管内皮细胞;手足口病;诊断价值

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Analysis of the Changes of Serum Levels of Ghrelin, β -EP and BMEC in Hand-foot-mouth Disease and Their Diagnostic Value*

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ABSTRACT Objective: To study Analysis of the Changes of serum levels of Growth hormone releasing peptide (Ghrelin), β -endorphin (β -EP), brain microvascular endothelial cells (BMEC) in hand-foot-mouth disease and their diagnostic value. **Methods:** 150 HFMD patients treated in our hospital from June 2017 to October 2019 were selected as the study group, and a total of 120 children with physical examination in the Department of Children's Health Care in our hospital were selected as the control group. The changes of serum levels of Ghrelin, β -EP and BMEC and their predictive value were analyzed. **Results:** The serum ghrelin level in the study group was significantly lower than that in the control group, and the levels of β -EP and BMEC were significantly higher than those in the control group, with significant differences ($P<0.05$). The serum ghrelin of stage II patients was significantly higher than that of stage III and IV patients, the serum β -EP and BMEC of stage III patients were significantly lower than that of stage III and IV patients, the serum ghrelin of stage III patients was significantly higher than that of stage IV patients, the serum β -EP and BMEC of stage III patients were significantly lower than that of stage IV patients, the difference was significant ($P<0.05$). ROC results showed that the AUC of serum ghrelin in predicting HFMD was 0.955, the sensitivity was 85.62%, the specificity was 87.45%, and the cutoff value was 12.91 ng/mL. The AUC of serum β -EP for predicting HFMD was 0.801, the sensitivity was 82.25%, the specificity was 83.56%, and the cut-off value was 196.87 ng/L. The AUC of serum BMEC in predicting HFMD was 0.974, the sensitivity was 84.18%, the specificity was 85.97%, and the cutoff value was 56.28 /mL. The combined detection had higher sensitivity and specificity than the single detection ($P<0.05$). **Conclusion:** Serum Ghrelin, β -EP and BMEC are related to the condition of patients with hand, foot and mouth disease, which is helpful to guide clinical diagnosis and treatment.

Key words: Growth hormone releasing peptide; β -endorphins; Cerebral microvascular endothelial cells; Hand, foot and mouth disease; Diagnostic value

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前言

手足口病是由肠道病毒 71 型和柯萨奇病毒 A 组 16 型等多种肠道病毒引起的,多发生于夏秋季,其中 3 岁以下年龄段发病率最高,主要表现为发热、手足和口腔出现疱疹,若得不到及时治疗可在短时间内迅速恶化,出现脑干脑炎、神经源性肺水肿等并发症,成为重症病例,严重危及患儿的生命^[1-3]。由于重症病例表现无特异性,易出现误诊、漏诊的情况,因此对于疾病的早期诊断已成为手足口病的迫切需要^[4,5]。血清 ghrelin 是一种内源性脑肠肽,在全身炎症反应下,能抑制炎症反应的发展,可对机体各器官产生保护的作用^[6]。β-EP 是一种能刺激情绪并使人产生愉悦感觉的化学物质,在多种疾病中表达较高;BMEC 脑是人体最复杂的器官,血脑屏障可维持脑内环境的稳定,而手足口病的发生可导致血脑屏障损伤,直接表现为 BMEC 损伤,导致细胞失去正常形态^[7-9]。目前对于关于小儿手足口病多以治疗方面的研究为主,对于早期诊断方面研究报道较少^[10]。因此,本研究旨在探讨血清 ghrelin、β-EP、BMEC 在手足口病中的水平变化,探讨其对临床的指导价值。

1 资料与方法

1.1 一般资料

选择 2017 年 6 月至 2019 年 10 月于我院进行治疗的 150 例手足口病患者进行研究,设为研究组,男 85 例,女 65 例,年龄 6 月~5 岁,平均(2.98±0.56)岁,根据临床分期分为 II 期 53 例、III 期 64 例、IV 期 33 例;选择我院 120 例儿童保健科查体

患儿作为对照组,男 78 例,女 42 例,年龄 30~63 岁,平均(48.47±3.08)岁。两组患者在年龄等一般资料无明显差异,具有可比性。

参照《手足口病诊疗指南》^[11]中的诊断标准;临床合并手足口皮疹;体温升高。

纳入标准:(1)符合相关标准;(2)患儿监护人知情同意;(3)相关指标数据完整;(4)知情同意。排除标准:(1)其他原因导致皮肤病者;(2)合并其他感染者;(3)近 3 个月有严重创伤或者手术;(4)严重自身免疫性疾病;(5)先天缺陷者。

1.2 方法与评价标准

采集两组对象入组后第 2 d 清晨空腹静脉血,以 3000 r·min⁻¹ 的速度进行离心,离心半径 10 cm,时间 10 min,提取上层血清后,置于零下 20℃ 的冷冻箱内存储以备检测,采用酶联免疫吸附法测定血清 ghrelin、β-EP、BMEC 水平,试剂盒由深圳晶美生物技术有限公司生产,仪器均使用东芝 GA800 生化分析仪,操作严格按试剂盒说明进行。

1.3 统计学分析

以 SPSS 22.0 软件包处理,计量资料用均数±标准差($\bar{x} \pm s$)表示,t 检验,使用受试者工作特征曲线(ROC)分析血清 ghrelin、β-EP、BMEC 的预测价值, $P < 0.05$ 为差异具有统计学意义。

2 结果

2.1 两组血清 ghrelin、β-EP、BMEC 检查结果比较

研究组血清 ghrelin 水平显著低于对照组,β-EP、BMEC 水平显著高于对照组,差异显著($P < 0.05$)见表 1。

表 1 两组血清 ghrelin、β-EP、BMEC 检查结果比较($\bar{x} \pm s$)

Table 1 Serum ghrelin, ghrelin β- Comparison of EP and BMEC results($\bar{x} \pm s$)

Groups	n	Ghrelin (ng/mL)	β-EP (ng/L)	BMEC (个/mL)
Research Group	150	11.29±4.12	220.29±37.15	93.25±21.56
Control group	120	25.64±6.85	176.67±31.92	47.61±10.93
t value		21.295	10.198	21.115
P value		0.000	0.000	0.000

2.2 不同分期血清 ghrelin、β-EP、BMEC 检查结果比较

II 期患儿血清 ghrelin 显著高于 III 期、IV 期患者,血清 β-EP、BMEC 显著低于 III 期、IV 期患者,III 期血清 ghrelin 显

著高于 IV 期患者,血清 β-EP、BMEC 显著低于 IV 期患者,差异显著($P < 0.05$)见表 2。

表 2 不同分期血清 ghrelin、β-EP、BMEC 检查结果比较($\bar{x} \pm s$)

Table 2 Serum ghrelin in different stages β- Comparison of EP and BMEC results($\bar{x} \pm s$)

Groups	n	Ghrelin (ng/mL)	β-EP (ng/L)	BMEC (Entries/mL)
IIperiod	53	15.52±4.13	200.15±35.69	72.56±20.85
IIIperiod	64	10.67±4.25	220.07±38.25	93.18±21.63
IVperiod	33	5.69±1.36	253.06±40.16	126.62±25.94
F value		70.809	19.927	59.345
P value		0.000	0.000	0.000

2.3 血清 ghrelin、β-EP、BMEC 预测手足口病的价值分析

ROC 结果显示,血清 ghrelin 预测手足口病的 AUC 为

0.955,灵敏度为 85.62%,特异度为 87.45%,截断值为 12.91 ng/mL;血清 β-EP 预测手足口病的 AUC 为 0.801,灵敏度

82.25%, 特异度为 83.56%, 截断值为 196.87 ng/L; 血清 BMEC 预测手足口病的 AUC 为 0.974, 灵敏度为 84.18%, 特异度为 85.97%, 截断值为 56.28 个 /mL, 联合检测较单独检测灵敏度、特异度更高 ($P < 0.05$) 见图 1、表 3。

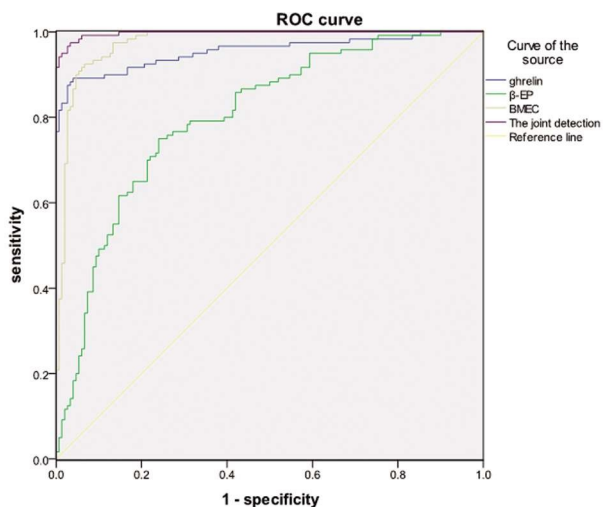


图 1 血清 ghrelin、β-EP、BMEC 预测手足口病的 ROC 曲线

Fig.1 ROC curve of serum ghrelin, β-EP and BMEC in predicting HFMD

表 3 血清 ghrelin、β-EP、BMEC 预测手足口病的价值分析

Table 3 Value analysis of serum ghrelin, β-EP and BMEC in predicting hand, foot and mouth disease

Project	AUC (95%CI)	Standard error	P	The sensitivity	Specific degrees	Cutoff value
Ghrelin	0.955 (0.928~0.982)	0.014	0.000	85.62	87.45	12.91 ng/mL
β-EP	0.801 (0.749~0.854)	0.027	0.000	82.25	83.56	196.87 ng/L
BMEC	0.974 (0.957~0.991)	0.009	0.000	84.18	85.97	56.28 个 /mL
The joint detection	0.987 (0.975~0.999)	0.002	0.000	93.56	94.58	-

带来严重危害, 影响患者恢复, 因此, 抑制炎症因子的释放在手足口病的治疗中具有重要意义^[20,21]。血清 ghrelin 是一种由 26~28 个氨基酸组成的内源性脑肠肽, 是新近发现的一种肠肽类激素, 广泛分布于中枢神经系统和外周器官组织, 能刺激生长素的释放, 调节能量代谢, 在炎症反应状态下对机体器官具有保护作用^[2]。有研究显示, ghrelin 能促进生长激素分泌, 参与能量平衡, 影响机体发育^[23]。Hoffmann A J^[24]等研究也显示, ghrelin 能通过抑制小胶质细胞炎症增生, 减少炎症因子的表达, 从而起保护神经元的退行性病变。本研究结果显示, 手足口病患儿血清 ghrelin 低于健康人, II 期患儿血清 ghrelin 显著高于 III 期、IV 期患者, III 期血清 ghrelin 显著高于 IV 期患者, 提示, 血清 ghrelin 在手足口病中呈低表达, 随着疾病分期而降低, 可作为预测疾病严重程度的标志物。分析其原因可能是因为手足口病的发生可引起机体免疫紊乱, 促进炎症细胞异常分泌, 引起炎症反应, ghrelin 则能上调凋亡蛋白 Bcl-2 表达, 抑制炎症介质的释放, 对机体各组织具有保护作用, 而随机疾病的进展, 而 ghrelin 水平降低代表对各器官的保护作用减弱, 从而随着疾病的严重程度而降低。Xuemin^[25]等研究也显示, ghrelin 作为体内重要的脑肠肽, 能在一定程度上抑制炎症反应的发生。

β-EP 是一种神经肽, 能调节中枢神经系统, 其水平增加可加重脑损伤等神经系统损害, 松弛血管平滑肌, 增加血管通透

3 讨论

手足口病是儿童肠道急性传染病, 是我国法定传染病报告管理中的丙类传染病, 根据病情的发展过程, 将手足口病分期分为 I 期、II 期、III 期、IV 期, 其中 I 期大多数病例在此期痊愈, II 期属于重症病例, 主要导致神经系统受累; III、IV 期属于重症病例危重型, 可导致心肺功能衰竭衰竭^[12-14]。近年来其发病明显升高, 引起人们关注, 患者主要表现为口痛、手、足、口腔等部位出现小疱疹, 多数患者在 1 周左右可自愈, 但仍有少数患者病情迅速恶化, 在数小时内并发脑膜炎、神经源性肺水肿等并发症, 进展为重症病例^[15,16]。有研究显示, 重症手足口病患者预后恶化更明显, 病死率超过 15%, 严重威胁患者生命^[17]。流行病学调查显示, 2008 年~2016 年我国共计发生 132757 例重症病例, 其中 3536 例死亡, 因此寻找特异性的生物标志物对手足口病诊断作为参考对病情的评估具有重要价值^[18]。白细胞、心肌酶升高等可作为手足口病的实验室指标, 但缺乏特异性, 尚未有一些检验指标敏感及特异地早期诊断重症手足口病^[19]。

有研究显示, 当机体被感染后, NK 细胞限制其入侵, T 细胞被激活, 并释放大量细胞因子, 而炎症因子分泌过多, 给机体

性, 同时还能抑制脑和肺内血管紧张素转换酶活性, 导致血管紧张素水平下降^[26]。有研究显示, β-EP 参与机体应激反应的发生, 当应激反应强烈时可激发下丘脑 - 垂体 - 肾上腺轴系统, 而造成神经内分泌系统紊乱^[27]。本研究结果显示, 手足口病患儿血清 β-EP 高于健康人, II 期患儿血清 β-EP 显著低于 III 期、IV 期患者, III 期血清 β-EP 显著低于 IV 期患者, 提示, 血清 β-EP 在手足口病中呈高表达, 可作为预测疾病的标志物。分析其原因可能是因为手足口病多是由于肠道病毒 71 型病毒感染引起的, 该病毒具有嗜神经性, 可对危害患者中枢神经系统, 而中枢神经系统在感染下可导致大脑释放 β-EP, 进入血液后通过下丘脑外侧直接进入脑脊液, 因此, β-EP 在手足口病中表达较高。BMEC 是血脑屏障的主要组成成分, 能反映脑血管内皮细胞的损伤程度和脑血管损伤的潜在风险^[28]。本研究结果显示, 手足口病患儿血清 BMEC 高于健康人, II 期患儿血清 BMEC 显著低于 III 期、IV 期患者, III 期血清 BMEC 显著低于 IV 期患者, Zhang J^[29]等研究也显示, 血脑屏障损伤越严重, 越多 BMEC 进入外周血中, 导致其水平升高。分析其原因可能是因为手足口病病情加重过程中, 可增加体内肠道病毒载量, 使其通过血脑屏障造成脑组织损伤, 最终导致 BMEC 水平升高。本研究进一步 ROC 分析结果显示, 血清 ghrelin 预测手足口病的 AUC 为 0.955, 灵敏度为 85.62%, 特异度为 87.45%, 截断值

为 12.91 ng/mL; 血清 β -EP 预测手足口病的 AUC 为 0.801, 灵敏度 82.25%, 特异度为 83.56%, 截断值为 196.87 ng/L; 血清 BMEC 预测手足口病的 AUC 为 0.974, 灵敏度为 84.18%, 特异度为 85.97%, 截断值为 56.28 个 /mL, 且联合检测较各指标单独检测灵敏度、特异度更高。

综上所述, 血清 ghrelin、 β -EP、BMEC 在手足口病中变化异常, 对病情评估及早期预测具有重要意义。

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