

doi: 10.13241/j.cnki.pmb.2020.05.028

二甲双胍联合枸橼酸氯米芬对多囊卵巢综合征不孕患者性激素和胰岛素水平的影响*

毕艳慧 高新源 向阳 胡晓文 潘巧玲

(北京中医药大学深圳医院妇科 广东 深圳 518121)

摘要 目的:探讨二甲双胍、枸橼酸氯米芬联合治疗对多囊卵巢综合征(PCOS)不孕患者性激素和胰岛素水平的影响。**方法:**选取我院于2017年1月到2018年7月期间收治的101例PCOS不孕患者,采用数字表法将患者随机分为对照组($n=50$)和研究组($n=51$),对照组给予枸橼酸氯米芬治疗,研究组在对照组基础上联合二甲双胍治疗,采用门诊复查等方式随访6个月,记录两组患者排卵率及妊娠率,比较两组患者治疗前、治疗后的性激素、胰岛素以及血管活性因子水平,记录不良反应发生情况。**结果:**两组患者治疗后黄体生成素(LH)、睾酮(T)、胰岛素(INS)、LH/卵泡刺激素(FSH)均较治疗前降低,且研究组低于对照组($P<0.05$)。研究组排卵率、妊娠率均高于对照组($P<0.05$)。两组患者治疗后血管紧张素(AT-II)、血管内皮生长因子(VEGF)均较治疗前降低,且研究组低于对照组($P<0.05$)。两组不良反应发生率对比无统计学差异($P>0.05$)。**结论:**二甲双胍联合枸橼酸氯米芬治疗PCOS不孕,安全有效,可有效调节患者胰岛素、性激素水平,提高排卵率、妊娠率,改善血管活性因子水平。

关键词:二甲双胍;枸橼酸氯米芬;多囊卵巢综合征;不孕;性激素;胰岛素

中图分类号:R711.75;R711.6 **文献标识码:**A **文章编号:**1673-6273(2020)05-927-04

Effects of Metformin Combined with Clomiphene Citrate on Sex Hormone and Insulin Levels in Infertile Women with Polycystic Ovary Syndrome*

BI Yan-hui, GAO Xin-yuan, XIANG Yang, HU Xiao-wen, PAN Qiao-ling

(Department of Gynaecology, Shenzhen Hospital, Beijing University of Chinese Medicine, Shenzhen, Guangdong, 518121, China)

ABSTRACT Objective: To investigate the effects of metformin combined with clomiphene citrate on sex hormone and insulin levels in infertile women with polycystic ovary syndrome (PCOS). **Methods:** 101 infertile patients with PCOS who were admitted to our hospital from January, 2017 to July 2018 were selected, and they were divided into control group ($n=50$) and study group ($n=51$) according to random number table method. The control group was treated with clomiphene citrate. The study group was treated with metformin on the basis of the control group. The patients were followed up for 6 months by outpatient reexamination, and the ovulation rate and pregnancy rate of the two groups were recorded. The sex hormone, insulin and vasoactive factor levels before and after treatment were compared between the two groups, and adverse reactions during treatment were recorded. **Results:** The luteinizing hormone (LH), testosterone (T), insulin (INS), LH/follicle stimulating hormone (FSH) in the two groups after treatment were lower than those before treatment, and those in the study group were lower than those in the control group ($P<0.05$). The ovulation rate and pregnancy rate in the study group were higher than those in the control group ($P<0.05$). Angiotensin (AT-II) and vascular endothelial growth factor (VEGF) were lower in the two groups after treatment than before treatment, and those in the study group were lower than those in the control group ($P<0.05$). There was no significant difference in the incidence of adverse reactions between the two groups ($P>0.05$). **Conclusion:** Metformin combined with clomiphene citrate is safe and effective in the treatment of PCOS infertility. It can effectively regulate the levels of serum sex hormones and insulin, improve ovulation rate, pregnancy rate and vasoactive factors in patients with PCOS infertility.

Key words: Metformin; Clomiphene citrate; Polycystic ovary syndrome; Infertility; Sex hormone; Insulin

Chinese Library Classification(CLC): R711.75; R711.6 **Document code:** A

Article ID: 1673-6273(2020)05-927-04

前言

多囊卵巢综合征(Polycystic ovary syndrome, PCOS)是临床常见的女性疾病,以慢性无排卵以及高雄激素血症为主要病理特征^[1,2]。PCOS常伴有不孕,据统计,约有60%的PCOS患者因无排卵而导致不孕,已成为育龄期妇女不孕的主要原因

之一^[3]。现临床上治疗PCOS不孕患者主要以纠正患者内分泌、促排卵为主,枸橼酸氯米芬可通过占据雌激素受体,进而抑制雌激素的负反馈作用,诱导卵泡发育成熟,是治疗PCOS不孕患者的一线治疗药物^[4,5]。然而不少临床实践证实^[6,7],单纯使用枸橼酸氯米芬易产生枸橼酸氯米芬抵抗,出现毒副反应,降低妊娠率。二甲双胍作为对胰岛素敏感的药物,不仅可增强胰岛

* 基金项目:广东省科技计划项目(2014A050512120)

作者简介:毕艳慧(1981-),女,本科,主治医师,研究方向:妇科学, E-mail: huihui_8606@sohu.com

(收稿日期:2019-07-06 接受日期:2019-07-30)

素敏感性、增强周围组织对糖的摄入,还可纠正 PCOS 患者高雄激素血症,促进卵巢排卵功能恢复,使患者恢复正常月经^[8,9]。本研究通过对我院收治的 PCOS 不孕患者给予二甲双胍联合枸橼酸氯米芬治疗,疗效满意,现报道如下。

1 资料与方法

1.1 基线资料

选取 2017 年 1 月到 2018 年 7 月我院接收的 PCOS 不孕患者 101 例,本次研究已经过我院伦理委员会批准进行。纳入标准:(1)均符合《多囊卵巢综合征诊治标准专家共识》^[10]中的相关诊断标准;(2)月经异常,持续无排卵,经检查患者输卵管畅通,配偶精液分析正常者;(3)患者及其家属知情并签署同意书;(4)近 3 个月内未经促排卵或激素类药物药物治疗者。排除标准:(1)合并其他内分泌紊乱性疾病者;(2)输卵管畸形者;(3)未按医嘱服药,无法判定疗效者;(4)对本次研究使用药物存在禁忌症者;(5)合并其他严重疾病且不易妊娠者;(6)失访者。根据数字表法将患者随机分为对照组($n=50$)和研究组($n=51$),其中对照组年龄 22~35 岁,平均(28.15 ± 2.39)岁;不孕时间 1~6 年,平均(3.29 ± 0.85)年;体质量指数 $20.2 \sim 24.8 \text{ kg/m}^2$,平均(22.17 ± 0.65) kg/m^2 。研究组年龄 23~36 岁,平均(28.29 ± 2.41)岁;不孕时间 1~7 年,平均(3.33 ± 0.67)年;体质量指数 $20.8 \sim 25.4 \text{ kg/m}^2$,平均(22.49 ± 0.83) kg/m^2 。两组患者基线资料对比无差异($P>0.05$),均衡可比。

1.2 治疗方法

对照组给予枸橼酸氯米芬(西南药业股份有限公司,国药准字 H50020039,规格:50 mg)治疗,服药方式如下:无月经者可任意一天开始服药,有月经者自经期第 5d 开始服药,口服,50 mg/次,1 次/d,连服 5d,每天同一时间点服药,以 3 个月经

周期为 1 个治疗疗程。研究组则在对照组基础上给予二甲双胍(迪沙药业集团有限公司,国药准字 H20103615,规格:0.25 g)治疗,口服,500 mg/次,3 次/d,以 3 个月经周期为 1 个治疗疗程。两组患者均治疗 1 个疗程。

1.3 观察指标

采用门诊复查等方式随访 6 个月,记录两组患者排卵率及妊娠率。于治疗前、治疗后采集患者清晨空腹静脉血 5 mL,以离心半径 6 cm,3200 r/min 的速率离心 12 min,分离血清,置于 -70°C 冰箱中待测。采用化学发光法检测黄体生成素(Luteinizing hormone, LH)、睾酮 (Testosterone, T)、卵泡刺激素(Follicle stimulating hormone, FSH)、胰岛素 (Insulin, INS)水平,并计算 LH/FSH 比值,试剂盒购自武汉博士德生物科技有限公司;采用放射免疫法检测血管紧张素(Angiotensin- II, AT- II)、血管内皮生长因子(Vascular endothelial growth factor, VEGF)水平,试剂盒购自上海基免实业有限公司,严格遵守试剂盒说明书。记录两组不良反应发生情况。

1.4 统计学方法

采用 SPSS20.0 软件进行统计分析。计数资料以百分比(%)的形式表示,采用 χ^2 检验。计量资料以均值 $\pm$ 标准差($\bar{x} \pm s$)的形式表示,采用 t 检验,以 $\alpha=0.05$ 为检验水准。

2 结果

2.1 性激素、胰岛素水平比较

两组治疗前 LH、T、FSH、INS、LH/FSH 比较无统计学差异($P>0.05$),两组患者治疗后 LH、T、INS、LH/FSH 均较治疗前降低,且研究组低于对照组($P<0.05$),两组患者治疗前、治疗后 FSH 比较差异均无统计学意义($P>0.05$),详见表 1。

表 1 两组患者性激素、胰岛素水平比较($\bar{x} \pm s$)

Table 1 Comparison of sex hormone and insulin levels in two groups($\bar{x} \pm s$)

Groups	LH(U/L)		T(nmol/L)		FSH(U/L)		INS(U/L)		LH/FSH	
	Before treatment	After treatment	Before treatment	After treatment	Before treatment	After treatment	Before treatment	After treatment	Before treatment	After treatment
Control group($n=50$)	10.35 $\pm$ 2.26	9.15 $\pm$ 1.63*	2.28 $\pm$ 0.38	1.77 $\pm$ 0.31*	5.18 $\pm$ 0.32	5.09 $\pm$ 0.34	40.89 $\pm$ 9.15	28.52 $\pm$ 7.06*	2.00 $\pm$ 0.33	1.80 $\pm$ 0.23*
Study group($n=51$)	10.46 $\pm$ 2.41	5.76 $\pm$ 1.34*	2.26 $\pm$ 0.41	1.39 $\pm$ 0.22*	5.17 $\pm$ 0.35	5.08 $\pm$ 0.51	41.31 $\pm$ 10.14	19.39 $\pm$ 6.79*	2.02 $\pm$ 0.36	1.13 $\pm$ 0.38*
t	0.237	11.427	0.254	2.136	0.150	0.116	0.218	6.625	0.436	10.534
P	0.814	0.000	0.800	0.035	0.881	0.908	0.828	0.000	0.664	0.000

Note: Compared with before treatment, * $P<0.05$.

2.2 两组患者排卵率妊娠率比较

随访期间,对照组排卵率、妊娠率分别为 46.00%(23/50)、22.00%(11/50),研究组排卵率、妊娠率分别为 66.67%(34/51)、49.02%(25/51),研究组排卵率、妊娠率均高于对照组($\chi^2=4.386$, 8.043 , $P=0.036$, 0.004)。

2.3 两组患者血管活性因子水平比较

两组治疗前 AT- II、VEGF 比较无统计学差异($P>0.05$),两组治疗后 AT- II、VEGF 均较治疗前降低,且研究组低于对照组($P<0.05$),详见表 2。

2.4 两组治疗期间不良反应发生情况

对照组用药期间发生恶心呕吐 2 例、乳房胀痛 2 例、头晕乏力 4 例,不良反应发生率为 16.00%(8/50);研究组用药期间发生恶心呕吐 3 例、乳房胀痛 3 例、头晕乏力 4 例、轻度腹泻 1 例,不良反应发生率为 21.57%(11/51);两组不良反应发生率对比无差异($\chi^2=0.519$, $P=0.474$)。

3 讨论

目前临床有关 PCOS 的发病机制尚不十分明确,其发病机

表 2 两组患者血管活性因子水平比较($\bar{x} \pm s$)Table 2 Comparison of the levels of vasoactive factor between the two groups($\bar{x} \pm s$)

Groups	AT-II (ng/L)		VEGF(ng/L)	
	Before treatment	After treatment	Before treatment	After treatment
Control group(n=50)	1.69± 0.23	1.42± 0.33*	0.36± 0.06	0.28± 0.05*
Study group(n=51)	1.67± 0.32	1.19± 0.29*	0.38± 0.07	0.21± 0.03*
t	0.360	3.723	1.540	8.551
P	0.720	0.000	0.127	0.000

Note: Compared with before treatment, * $P < 0.05$.

制复杂,一般认为与下丘脑-垂体-卵巢轴功能失常、遗传、肾上腺功能紊乱、代谢、生理、环境等因素有关^[11-13]。由于PCOS会导致一系列的临床症状,如胰岛素出现抵抗者会引发糖尿病、代偿性高胰岛素血症等代谢并发症,而雄性激素过量则可使患者出现不孕、多毛、痤疮等并发症,其中大部分PCOS患者均处于卵巢功能不全状态,出现排卵减少或不排卵,产生不孕现象^[14-16]。枸橼酸氯米芬是一种三苯乙炔叔胺类化合物,可对雌激素发挥弱激动和强拮抗等双重作用,其对体内有一定雌激素水平的PCOS不孕患者具有一定的疗效^[17,18]。但既往相关学者研究表明^[19],PCOS患者若是胰岛素水平正常者,其对枸橼酸氯米芬的敏感性较好,但机体高胰岛素血症可降低患者对枸橼酸氯米芬的敏感性,引起排卵效果不佳。李圣贤等人^[20]研究报道亦表明,高胰岛素血症参与着PCOS的生理病理发展过程,也是导致患者不孕的重要因素之一。二甲双胍是临床治疗糖尿病的常用药,可调节机体雄性激素、胰岛素并促进卵泡发育^[21-23]。

本次研究结果表明,研究组排卵率、妊娠率均高于对照组,可见二甲双胍联合枸橼酸氯米芬治疗可进一步改善患者排卵率、妊娠率,虽然枸橼酸氯米芬可提高患者排卵率,但其抑制了雌激素的作用,阻碍了其对于子宫内膜的刺激,降低胚胎着床率,减少了妊娠率^[24]。二甲双胍的促排卵为渐进式,可弥补枸橼酸氯米芬纠正PCOS内分泌的不足,恢复患者卵巢功能,促进妊娠率^[25]。由于PCOS不孕是一种多原因产生的内分泌紊乱综合征,故其尚无公认的生化指标,但内分泌激素如LH、T、INS、LH/FSH等水平变化是重要的临床特征。T是女性体内重要的雄性激素,正常女性含量较低,在PCOS不孕患者中,T分泌过高,可影响PCOS患者受孕及胚胎着床^[26]。INS可通过胰岛素样生长因子放大促性腺激素的作用,引起卵泡大量募集,引发卵巢多囊样改变^[27]。PCOS不孕患者体内LH水平升高是由多种因素引起的继发性调节障碍,过高的LH可引起卵泡的过早成熟,进而影响卵泡质量及胚胎细胞^[28]。此外,LH/FSH的比值升高时,同样可提示机体存在不同程度的排卵障碍^[29]。两组治疗后LH、T、INS、LH/FSH均降低,且研究组低于对照组,可见二甲双胍联合枸橼酸氯米芬可更好的调节患者性激素及胰岛素水平,枸橼酸氯米芬有着与雌激素相似的结构,可竞争性抑制雌激素,且其与雌激素受体结合时间相对较长,可发挥较长的作用时间,反射性促进卵泡发育,加之二甲双胍可有效降低胰岛素水平,提高下丘脑垂体对枸橼酸氯米芬的敏感性,恢复卵巢功能,进而改善相关性激素指标^[30]。同时研究组治疗后AT-II、VEGF低于对照组,可见联合治疗可有效降低血管活性因

子水平,抑制卵巢血管形成,进而促进排卵。此外,两组不良反应发生率对比差异无统计学意义,可见上述联合治疗安全性较好,不会增加机体毒副作用。

综上所述,二甲双胍联合枸橼酸氯米芬治疗PCOS不孕患者,在改善血清性激素、胰岛素、排卵率、妊娠率以及血管活性因子等方面效果显著,不会增加毒副作用,临床应用价值较高。

参考文献(References)

- [1] Li J, Xie LM, Song JL, et al. Alterations of Sphingolipid Metabolism in Different Types of Polycystic Ovary Syndrome[J]. Sci Rep, 2019, 9(1): 3204
- [2] 李扬璐,阮祥燕, Alfred O. Mueck, 等. 多囊卵巢综合征对妊娠结局的影响研究进展[J]. 首都医科大学学报, 2016, 37(4): 449-453
- [3] 王文娟,吴海波,杨琴,等. PCOS患者外周血白细胞计数变化的临床意义[J]. 现代生物医学进展, 2016, 16(31): 6118-6120
- [4] Niederberger C. Re: Effects of Clomiphene Citrate on Male Obesity-Associated Hypogonadism[J]. J Urol, 2019, 201(3): 427-428
- [5] Eskew AM, Bedrick BS, Hardi A, et al. Letrozole Compared With Clomiphene Citrate for Unexplained Infertility: A Systematic Review and Meta-analysis[J]. Obstet Gynecol, 2019, 133(3): 437-444
- [6] Yu Q, Hu S, Wang Y, et al. Letrozole versus laparoscopic ovarian drilling in clomiphene citrate-resistant women with polycystic ovary syndrome: a systematic review and meta-analysis of randomized controlled trials[J]. Reprod Biol Endocrinol, 2019, 17(1): 17
- [7] 许海,马丹凤,赵井苓,等. 艾塞那肽联合枸橼酸氯米芬治疗多囊卵巢综合征伴胰岛素抵抗的临床观察 [J]. 中国药房, 2017, 28(12): 1606-1609
- [8] Abbott DH. Metformin use in polycystic ovary syndrome pregnancy impacts on offspring obesity[J]. Lancet Child Adolesc Health, 2019, 3(3): 132-134
- [9] 蔡艳悦,金敏娟. 探讨多囊卵巢综合征采用炔雌醇环丙孕酮片联合二甲双胍治疗疗效及其对胰岛素抵抗、激素影响[J]. 中国生化药物杂志, 2016, 36(3): 67-69
- [10] 郝琦. 多囊卵巢综合征诊治标准专家共识 [J]. 中国实用妇科与产杂志, 2007, 23(6): 474
- [11] Kite C, Lahart IM, Afzal I, et al. Exercise, or exercise and diet for the management of polycystic ovary syndrome: a systematic review and meta-analysis[J]. Syst Rev, 2019, 8(1): 51
- [12] Dragamestianos C, Messini CI, Antonakis PT, et al. The Effect of Metformin on the Endometrium of Women with Polycystic Ovary Syndrome[J]. Gynecol Obstet Invest, 2019, 84(1): 35-44
- [13] Marchesan LB, Spritzer PM. ACC/AHA 2017 definition of high

- blood pressure: implications for women with polycystic ovary syndrome[J]. *Fertil Steril*, 2019, 111(3): 579-587.e1
- [14] Dawood AS, Elgergawy A, Elhalwagy A. Circulating Levels of Vitamin D3 and Leptin in Lean Infertile Women with Polycystic Ovary Syndrome[J]. *J Hum Reprod Sci*, 2018, 11(4): 343-347
- [15] Shi XY, Huang AP, Xie DW, et al. Association of vitamin D receptor gene variants with polycystic ovary syndrome: a meta-analysis [J]. *BMC Med Genet*, 2019, 20(1): 32
- [16] Januszewski M, Issat T, Jakimiuk AA, et al. Metabolic and hormonal effects of a combined Myo-inositol and d-chiro-inositol therapy on patients with polycystic ovary syndrome? (PCOS) [J]. *Ginekol Pol*, 2019, 90(1): 7-10
- [17] Rezk M, Shaheen AE, Saif El-Nasr I. Clomiphene citrate combined with metformin versus letrozole for induction of ovulation in clomiphene-resistant polycystic ovary syndrome: a randomized clinical trial[J]. *Gynecol Endocrinol*, 2018, 34(4): 298-300
- [18] Ma HL, Xie LZ, Gao JS, et al. Acupuncture and clomiphene for Chinese women with polycystic ovary syndrome (PCOSAct): statistical analysis approach with the revision and explanation [J]. *Trials*, 2018, 19(1): 601
- [19] 刘卫民, 柳学芳, 鲍友娥, 等. 痰脂消颗粒联合枸橼酸氯米芬治疗多囊卵巢综合征伴胰岛素抵抗 45 例临床研究[J]. *中国药业*, 2018, 27(14): 23-26
- [20] 李圣贤, 褚倩倩, 麻静, 等. 胰岛素和雄激素对多囊卵巢综合征患者血清脂质谱及活性脂质生成调节的初步研究[J]. *中华内分泌代谢杂志*, 2017, 33(8): 644-650
- [21] Stener-Victorin E, Zhang H, Li R, et al. Acupuncture or metformin to improve insulin resistance in women with polycystic ovary syndrome: study protocol of a combined multinational cross sectional case-control study and a randomised controlled trial[J]. *BMJ Open*, 2019, 9(1): e024733
- [22] Ou HT, Chen PC, Wu MH, et al. Correction to: Metformin improved health-related quality of life in ethnic Chinese women with polycystic ovary syndrome[J]. *Health Qual Life Outcomes*, 2018, 6(1): 217
- [23] Artani M, Iftikhar MF, Khan S. Effects of Metformin on Symptoms of Polycystic Ovarian Syndrome Among Women of Reproductive Age[J]. *Cureus*, 2018, 10(8): e3203
- [24] Takasaki A, Tamura I, Okada-Hayashi M, et al. Usefulness of intermittent clomiphene citrate treatment for women with polycystic ovariansyndrome that is resistant to standard clomiphene citrate treatment[J]. *Reprod Med Biol*, 2018, 17(4): 454-458
- [25] D'Amato G, Caringella AM, Stanziano A, et al. Mild ovarian stimulation with letrozole plus fixed dose human menopausal gonadotropin prior to IVF/ICSI for infertile non-obese women with polycystic ovarian syndrome being pre-treated withmetformin: a pilot study[J]. *Reprod Biol Endocrinol*, 2018, 16(1): 89
- [26] Abbasi-Ranjbar Z, Sharami SH, Kazemi S, et al. The Relation Between Free Testosterone and Components of Metabolic Syndrome in Women WithPolycystic Ovary Syndrome [J]. *J Family Reprod Health*, 2018, 12(1): 1-7
- [27] Morshedzadeh N, Saedisomeolia A, Djalali M, et al. Resolvin D1 impacts on insulin resistance in women with polycystic ovary syndrome and healthy women[J]. *Diabetes Metab Syndr*, 2019, 13(1): 660-664
- [28] Crisosto N, Ladrón de Guevara A, Echiburú B, et al. Higher luteinizing hormone levels associated with antimüllerian hormone in postmenarchal daughters of women with polycystic ovary syndrome [J]. *Fertil Steril*, 2019, 111(2): 381-388
- [29] 张慧, 王瑞玲, 李群英, 等. 二甲双胍与炔雌醇环丙孕酮治疗多囊卵巢综合征的疗效及对性激素、血糖水平的影响[J]. *疑难病杂志*, 2016, 15(6): 621-624
- [30] Erensoy H, Niafar M, Ghafarzadeh S, et al. A pilot trial of metformin for insulin resistance and mood disturbances in adolescent and adult women with polycystic ovary syndrome [J]. *Gynecol Endocrinol*, 2019, 35(1): 72-75

(上接第 926 页)

- [25] Thude H, Bischoff W, Sterneck M, et al. Polymorphisms of the human platelet antigen-1, -2, -3, -5, and -15 systems and acute cellular liver transplant rejection[J]. *Hum Immunol*, 2017, 78(9): 534-539
- [26] 李州利, 石炳毅, 蔡明, 等. 血小板 T 细胞活化抗原 1 与移植肾急性排斥反应的相关性 [J]. *肾脏病与透析肾移植杂志*, 2003, 12(3): 229-233, 239
- [27] Mohamed AB, Elnady HM, Alhewaig HK, et al. The mean platelet volume and plateletcrit as predictors of short-term outcome of acute ischemic stroke[J]. *Egypt J Neurol Psychiatr Neurosurg*, 2019, 55(1): 4
- [28] Pyo JS, Cho WJ. Mean Platelet Volume, Platelet Distribution Width, and Platelet Count in Varicocele: A Systematic Review and Meta-Analysis[J]. *Cell Physiol Biochem*, 2016, 38(6): 2239-2246
- [29] 孙永康. 肾移植患者血小板相关参数与急性排斥反应的相关性研究[J]. *中国基层医药*, 2018, 25(16): 2070-2073
- [30] Iesari S, Lai Q, Ruggetti A, et al. Infected Nonhealing Wound in a Kidney Transplant Recipient: Successful Treatment With Topical Homologous Platelet-Rich Gel [J]. *Exp Clin Transplant*, 2017, 15(2): 222-225