科技部補助專題研究計畫成果報告
期末報告

維生素B-6與穀胱甘肽的單獨及協同作用對肝功能損傷小鼠以及肝硬化及肝硬化合併肝癌患者的發炎反應、同半胱胺酸代謝、氧化壓力及抗氧化能力的影響(第3年)

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報 告 附 件 ： 出席國際學術會議心得報告

中 華 民 國 107 年 08 月 31 日

中 文 摘 要 ： 本研究目的探討：1)小鼠肝功能壞死過程中，維生素B-6及穀胱甘肽濃度與同半胱胺酸、胱胺酸、氧化壓力、氧化型穀胱甘肽及穀胱甘肽相關抗氧化酵素活性之相關性；2)小鼠肝功能壞死過程中，單獨或合併給予維生素B-6及穀胱甘肽，維生素B-6及穀胱甘肽濃度變化對同半胱胺酸、胱胺酸、氧化壓力、氧化型穀胱甘肽及穀胱甘肽相關抗氧化酵素活性之影響；3) 比較及觀察健康受試者、肝硬化患者的血漿維生素B-6及穀胱甘肽濃度與發炎反應指標、同半胱胺酸、胱胺酸、氧化壓力、氧化型穀胱甘肽及穀胱甘肽相關抗氧化酵素活性之差異性及關係；4) 單獨或合併給予維生素B-6及GSH的補充對肝硬化患者的發炎反應、同半胱胺酸、胱胺酸、氧化壓力、氧化型穀胱甘肽及穀胱甘肽相關抗氧化酵素活性之影響。動物試驗是以40隻BALB/c雄性小鼠隨機分成5組。第一組為控制組，一星期3次以管餵方式給予 0.9%生理食鹽水；第二組為APAP組，以低劑量的APAP一星期3次以管餵方式誘發小鼠肝損傷；第三組為APAP+B-6組，以低劑量的APAP一星期3次以管餵方式誘發小鼠肝損傷外，小鼠每天攝取維生素B-6補充飼料；第四組為APAP+GSH組，以低劑量的APAP一星期3次以管餵方式誘發小鼠肝損傷外，小鼠每天攝取GSH補充飼料；第五組則為APAP+B-6+GSH組，除了以低劑量的APAP一星期3次以管餵方式誘發小鼠肝損傷外，小鼠每天攝取維生素B-6及GSH補充飼料。於第4及8週，各組各犧牲4隻小鼠，取其血漿及肝組織。人體試驗是以醫院為基礎的橫斷面及3個月的隨機雙盲的補充劑介入研究。於臺中榮總一般外科募集肝硬化或肝硬化合併肝癌患者。同意參與介入研究的受試者被隨機分派至以下四組：1）安慰組）；2）維生素B-6組（50 mg/d）；3）GSH組（500 mg/d）；4）維生素B-6 + GSH組（50 mg/d維生素B-6 + 500 mg/d GSH)。另外於臺中榮總體檢科募集健康受試者。五組小鼠在第4週時的維生素B-6、同半胱胺酸、半胱胺酸、GSH、GSSG、GSH還原酶活性、氧化壓力指標以及總抗氧化能力的數值皆無顯著差異。在第8週時除了丙二醛濃度在B6+GSH組較其他組別顯著增加外，其餘肝臟組織的各項數值也無顯著變化。B-6以及B-6+GSH組的肝硬化受試者每天補充50 mg的維生素B-6後，其血漿PLP濃度較未補充前顯著上升但是GSH及B-6+GSH組的受試者雖每天補充500 mg的GSH，其血漿GSH濃度在整個補充劑介入期間並未有顯著變化。B-6組的GST活性在第12週時較第0週時顯著增加，但GPx活性卻顯著下降。B6+GSH組的總抗氧化能力在12週時較第0週顯著上升。研究結果指出維生素B-6及GSH的補充無法降低肝損傷小鼠或肝硬化患者的氧化壓力，也無法增加其抗氧化能力。

中 文 關 鍵 詞 ： 肝硬化、維生素B-6、穀胱甘肽、發炎反應、氧化壓力、抗氧化酵素活性

英 文 摘 要 ： This study was to: 1) assess the relationships of vitamin B-6 and GSH with homocysteine, oxidative stress, oxidized GSH (GSSG), GSH related antioxidant enzyme activities in mice with chronic hepatotoxicity; 2) evaluate the effects of individual or combined supplementation of vitamin B-6 and GSH on homocysteine, oxidative stress, GSSG and GSH related antioxidant enzyme activities in mice with chronic hepatotoxicity; 3) compare the differences and associations

among vitamin B-6, GSH, homocysteine, oxidative stress, GSSH and GSH related antioxidant enzyme activities in healthy subjects and patients with cirrhosis and cirrhosis combined with HCC; 4) assess the effects of individual or combined supplementation of vitamin B-6 and GSH on homocysteine, cysteine, oxidative stress, GSSG and GSH related antioxidant enzyme activities in patients with cirrhosis and cirrhosis combined with HCC. Forty BALB/c mice were divided into one of five groups: 1) a control group with 3 times a week tube feeding of 0.9% saline; 2) a APAP group with 3 times a week tube feeding of APAP; 3) a APAP + B-6 group with 3 times a week tube feeding of APAP and oral supplemented with 14 mg/diet of PN-HCl; 4) a APAP + GSH group with 3 times a week tube feeding of APAP and oral supplemented with 1 g/kg BW of GSH; 5) a APAP + B-6 + GSH group with 3 times a week tube feeding of APAP and oral supplemented with 14 mg/diet of PN-HCl and 1g/kg BW GSH. At the 4th and 8th wk, 4 mice of each group were sacrificed. Human study is designed as a cross-sectional and randomized double-blind placebo-controlled trial. Healthy controls were recruited from the Physical Check unit of TGVH. Patients with either cirrhosis or cirrhosis combined with HCC were recruited from TGVH. Patients were randomly assigned to either the 1) placebo group; 2) B-6 group; 3) GSH group (500 mg/d); or 4) B-6 (50 mg/d) plus GSH (500 mg/d) group for 3 mo. Patients who participated in the intervention study had blood drawn at month 0, 1, 2 and 3 during intervention period. There were no significant differences in vitamin B-6, homocysteine, cysteine, GSH, GSSG, GSH reductase activity, oxidative stress indicator and total antioxidant capacities among five mice groups at week 4. Only mice in the B-6+GSH group had significantly increased oxidative stress indicator when compared to other groups at week 4. Patients with liver cirrhosis had significant higher plasma pyridoxal 5' -phosphate concentration after 50 mg/d vitamin B-6 supplementation. However, 500 mg/d GSH supplementation did not increase plasma GSH concentration in GSH and B-6 + GSH groups. Plasma GSH S-transferase activity significantly increased at week 12, while GSH peroxidase activity significantly decreased at week 12 when compared to week 0. Total antioxidant capacity was significantly increased at week 12 when compared to levels at week 0 in B-6 + GSH group. The results of this study indicated vitamin B-6 or GSH supplementation could not decrease oxidative stress, and could not increase antioxidant capacity in mice or patients with liver cirrhosis.

英文關鍵詞：cirrhosis, vitamin B-6, glutathione, inflammatory responses, oxidative stress, antioxidant enzyme activities

科技部補助專題研究計畫成果報告

（□期中進度報告/■期末報告）

維生素 B-6 與穀胱甘肽的單獨及協同作用對肝功能損傷小鼠以及肝硬化及肝硬化合併肝癌患者的發炎反應、同半胱胺酸代謝、氧化壓力及抗氧化能力的影響

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中　華　民　國　107　年　8　月　31　日

中文摘要

維生素 B-6 與穀胱甘肽主要在肝臟代謝，若肝功能受損將影響維生素 B-6 與穀胱甘肽所擔任的抗發炎及抗氧化等的重要生理功能。本研究以三年時間利用動物及人體試驗模式探討以下目的：1) 在小鼠逐漸形成肝功能壞死過程中，血漿及組織維生素 B-6 及穀胱甘肽濃度與同半胱胺酸、胱胺酸、氧化壓力、氧化型穀胱甘肽及穀胱甘肽相關抗氧化酵素活性之相關性；2) 在小鼠逐漸形成肝功能壞死過程中，單獨或合併給予維生素 B-6 及穀胱甘肽的補充，血漿及組織維生素 B-6 及穀胱甘肽濃度變化對同半胱胺酸、胱胺酸、氧化壓力、氧化型穀胱甘肽及穀胱甘肽相關抗氧化酵素活性之影響；3) 比較及觀察健康受試者、肝硬化及肝硬化合併肝癌患者的血漿維生素 B-6 及穀胱甘肽濃度與發炎反應指標、同半胱胺酸、胱胺酸、氧化壓力、氧化型穀胱甘肽及穀胱甘肽相關抗氧化酵素活性之差異性及關係；4) 單獨或合併給予維生素 B-6 及 GSH 的補充對肝硬化及肝硬化合併肝癌患者的發炎反應指標、同半胱胺酸、胱胺酸、氧化壓力、氧化型穀胱甘肽及穀胱甘肽相關抗氧化酵素活性之影響。

動物試驗是以40隻BALB/c雄性小鼠入室適應後隨機分成5組。第一組為控制組，一星期3次以管餵方式給予 0.9%生理食鹽水；第二組為APAP組，以低劑量的APAP溶於生理食鹽水中，一星期3次以管餵方式誘發小鼠肝損傷；第三組為APAP + B-6組，除了以低劑量的APAP溶於生理食鹽水中，一星期3次以管餵方式誘發小鼠肝損傷外，此組小鼠每天攝取的是維生素B-6補充飼料(添加14 mg/diet of PN-HCl)；第四組為APAP + GSH組，除了以低劑量的APAP溶於生理食鹽水中，一星期3次以管餵方式誘發小鼠肝損傷外，此組小鼠每天攝取的是GSH補充飼料(添加1 g/kg BW of GSH)；第五組則為APAP + B-6 + GSH組，除了以低劑量的APAP溶於生理食鹽水中，一星期3次以管餵方式誘發小鼠肝損傷外，此組小鼠每天攝取的是維生素B-6及GSH補充飼料(添加14 mg/diet of PN-HCl 及1 g/kg BW of GSH)。之後於第4及8週，各組各犧牲4隻小鼠，取其血漿及肝組織。人體試驗是以醫院為基礎的橫斷面及隨機雙盲的補充劑介入研究。於臺中榮總一般外科募集肝硬化或肝硬化合併肝癌患者。若同意參與介入研究的受試者將被隨機分派至以下四組：1) 安慰組，n = 25）；2) 維生素B-6組（50 mg/d，n = 25）；3) GSH組（500 mg/d，n = 25）；4) 維生素B-6 + GSH組 (50 mg/d維生素B-6 + 500 mg/d GSH，n = 25)。另外於臺中榮總體檢科募集健康受試者。收集所有受試者的基本資料、體位測量及藥物疾病史的資料。肝硬化及肝硬化合併肝癌患者的空腹血液於門診時採集，健康受試者的空腹血液於體檢時採集，參加介入的患者的空腹血液則於介入前、介入第1、2及第3個月時採集。

五組小鼠在第 4 週時的維生素 B-6、同半胱胺酸、半胱胺酸、GSH、GSSG、GSH 還原酶活性、氧化壓力指標(丙二醛)以及總抗氧化能力的數值皆無顯著差異。在第 8 週時除了丙二醛濃度在 B6+GSH 組較其他組別顯著增加外，其餘肝臟組織的各項數值也無顯著變化。B-6 以及 B-6+GSH 組的肝硬化受試者每天補充 50 mg 的維生素 B-6 後，其血漿 PLP 濃度較未補充前顯著上升。但是 GSH 及 B-6+GSH 組的受試者雖每天補充 500 mg 的 GSH，其血漿 GSH 濃度在整個補充劑介入期間並未有顯著變化。B-6 組的 GST 活性在第 12 週時較第 0 週時顯著增加，但 GPx 活性卻顯著下降。B6+GSH 組的總抗氧化能力在 12 週時較第 0 週顯著上升。本研究結果指出維生素 B-6 及 GSH 的補充無法降低肝損傷小鼠或肝硬化患者的氧化壓力，也無法增加其抗氧化能力。

關鍵詞：肝硬化、維生素 B-6、穀胱甘肽、發炎反應、氧化壓力、抗氧化酵素活性

英文摘要

Liver cirrhosis is now the ninth leading cause of death and hepatocellular carcinoma (HCC) is the second leading cause of cancer mortality among men and women in Taiwan. Vitamin B-6 and glutathione (GSH) are metabolized in liver, the role of vitamin B-6 and GSH playing in the inflammatory responses and antioxidant function might be impaired during hepatic injury. The 3-y animal and human studies were to: 1) assess the relationships of vitamin B-6 and GSH with homocysteine, oxidative stress, oxidized GSH (GSSG), GSH related antioxidant enzyme activities in mice with chronic hepatotoxicity; 2) evaluate the effects of individual or combined supplementation of vitamin B-6 and GSH on homocysteine, oxidative stress, GSSG and GSH related antioxidant enzyme activities in mice with chronic hepatotoxicity; 3) compare and observe the differences and associations among vitamin B-6, GSH, homocysteine, oxidative stress, GSSH and GSH related antioxidant enzyme activities in healthy subjects and patients with cirrhosis and cirrhosis combined with HCC; 4) assess the effects of individual or combined supplementation of vitamin B-6 and GSH on homocysteine, cysteine, oxidative stress, GSSG and GSH related antioxidant enzyme activities in patients with cirrhosis and cirrhosis combined with HCC.

Forty 5-week old male BALB/c mice were weighted and evenly divided into one of five groups: 1) a control group with 3 times a week tube feeding of 0.9% saline; 2) a APAP group with 3 times a week tube feeding of APAP (400 mg/kg BW); 3) a APAP + vitamin B-6 group with 3 times a week tube feeding of APAP (400 mg/kg BW) and oral supplemented with 14 mg/diet of PN-HCl; 4) a APAP + GSH group with 3 times a week tube feeding of APAP (400 mg/kg BW) and oral supplemented with 1 g/kg BW of GSH; 5) a APAP + vitamin B-6 + GSH group with 3 times a week tube feeding of APAP (400 mg/kg BW) and oral supplemented with 14 mg/diet of PN-HCl and 1g/kg BW GSH. At the 4^{th} and 8^{th} wk, 4 mice of each group were sacrificed to obtain blood and tissue samples. Human study is designed as a hospital-based cross-sectional and randomized double-blind placebo-controlled intervention trial. Healthy controls were recruited from the Physical Check unit of Taichung General Veterans Hospital. Patients with either cirrhosis or cirrhosis combined with hepatocellular carcinoma who meet the inclusion criteria were recruited from Taichung General Veterans Hospital. Patients were randomly assigned to either the 1) placebo group; 2) vitamin B-6 group; 3) GSH group (500 mg/d); or 4) vitamin B-6 (50 mg/d) plus GSH (500 mg/d) group for 3 mo. Data on demography, anthropometry and medical history were collected. Fasting blood drawn were obtained. Patients who participated in the intervention study had blood drawn at month 0, 1, 2 and 3 during intervention period.

There were no significant differences in vitamin B-6, homocysteine, cysteine, GSH, GSSG, GSH reductase activity, oxidative stress indicator and total antioxidant capacities among five mice groups at week 4. Only mice in the B-6+GSH group had significantly increased oxidative stress indicator when compared to other groups at week 4. Patients with liver cirrhosis had significant higher plasma pyridoxal 5'-phosphate (PLP) concentration after 50 mg/d vitamin B-6 supplementation. However, 500 mg/d GSH supplementation did not increase plasma GSH concentration in GSH and B-6 + GSH groups. Plasma GSH S-transferase activity significantly increased at week 12, while GSH peroxidase activity significantly decreased at week 12 when compared to week 0. Total antioxidant capacity was significantly increased at week 12 when compared to levels at week 0 in B-6 + GSH group. The results of this study indicated vitamin B-6 or GSH supplementation could not decrease oxidative stress, and could not increase antioxidant capacity in mice or patients with liver cirrhosis.

Keywords: cirrhosis, vitamin B-6, glutathione, inflammatory responses, oxidative stress, antioxidant enzyme activities

背景

當肝臟受到病毒、藥物、內毒素及酒精等傷害時，肝細胞會因受傷而分泌細胞激素(cytokines)活化肝臟的庫氏細胞和星狀細胞，活化的庫氏細胞也會分泌一些細胞激素刺激星狀細胞活化而產生細胞外基質，當細胞外基質大量堆積時會造成纖維化，造成血管阻力增加，最後形成肝硬化。臨床上，大約有 65 ~ 80%的肝細胞癌(hepatocellular carcinoma，簡稱肝癌)是因為慢性發炎導致肝纖維化(advanced fibrosis)或肝硬化(cirrhosis)而造成 (Bosch et al., 2004; Sanhiovanni et al., 2004; Guyot et al., 2006)。根據衛生福利部的統計，肝硬化是台灣十大死因的第九名，而肝癌是台灣男性及女性主要癌症死亡原因的第二名(衛生福利部國民健康署，2018)。

當肝臟暴露在病毒、藥物、內毒素及酒精等環境下，若無法將這些物質代謝成不反應或非免疫原性(non-immunogenic)物質，這些物質不僅會造成肝細胞損傷也會趨使肝臟的庫氏細胞及星狀細胞釋放發炎細胞激素［如：介白素-1 (interleukin-1, IL-1)、介白素-6 (interleukin-6, IL-6)、介白素-8 (interleukin-8, IL-8)、腫瘤壞死因子-α (tumor necrosis factor-α, TNF-α)]，進而引起一連串的免疫及發炎反應(Stauffer et al., 2012)。在發炎反應過程中會增加活性氧（reactive oxygen species, ROS）及活性氮（reactive nitrogen species, RNS）的生成，使得體內氧化還原機制失去平衡，而增加體內氧化壓力(Valko et al., 2007 ; Stauffer et al., 2012)。ROS及RNS為氧分子及氮分子代謝後的產物，包括帶有未成對電子，當細胞外過多的ROS或RNS形成過高的氧化壓力環境，會造成基因調控失調及細胞傷害，因而導致細胞不正常的增生及肝癌細胞的形成（Severi et al., 2007）。急性肝炎(acute hepatitis)患者在急性期時的血清丙二醛(malondialdehyde, MDA)濃度(氧化壓力指標)顯著高於復原期時的濃度(Nagoev et al., 2002)。肝癌患者的MDA濃度也顯著高於健康受試者，但抗氧化酵素活性[穀胱甘肽過氧化氫酶(glutathione peroxidase, GPx)、 超氧化物岐化酶(superoxide oxidase, SOD)及觸酶(catalase, CAT)]卻顯著低於健康受試者(Lin & Yin, 2007;Yahya et al., 2013)。肝硬化及肝癌患者不僅有較高的血清MDA濃度外，肝臟組織的MDA濃度也顯著高於正常組織(Czeczot et al., 2006)。除了醫療介入之外，若能提供抗氧化營養素的補充以改善肝損傷或肝癌患者體內的抗氧化-氧化平衡機制，或許能預防或降低肝損傷而逐漸形成的肝硬化發生或肝癌的復發以及提高存活率。

維生素 B-6 主要以 3 種自由型式：比哆醇（pyridoxine; PN），比哆胺（pyridoxamine, PM）及比哆醛（pyridoxal, PL），及其磷酸鹽型式：磷酸比哆醇（pyridoxine 5’-phosphate, PNP），磷酸比哆胺（pyridoxamine 5’-phosphate; PMP）及磷酸比哆醛（pyridoxal 5’-phosphate, PLP）存在自然界。其中 PLP 是具有生物活性的輔酶。尿液比哆酸（4-pyridoxic acid; 4-PA）為維生素 B-6 的主要代謝產物。維生素 B-6 被腸道細胞吸收之後會在肝臟中進行代謝，此時不同型式的維生素 B-6 經磷酸激酶（pyridoxal kinase）轉換成磷酸化型式。肝臟維生素 B-6 代謝與血漿 PLP 濃度存在非常顯著的相關性。血漿 PLP 濃度受到肝臟合成及以及組織的降解作用所影響。維生素 B-6 是以 PLP 擔任輔酶，其醛基與胺基酸上的胺基反應，形成 shiff base，能轉移胺基酸上的電荷，參與轉胺作用、胺基酸代謝時的去羧作用、肝醣、比咯紫質、脂肪、cytokines、同半胱胺酸（homocysteine）及半胱胺酸（cysteine）的代謝，甚至間接參與還原型穀胱甘肽（reduced glutathione, GSH）抗氧化防禦機制。因此，PLP 若不足或缺乏將會影響單碳代謝、免疫功能及發炎反應、增加 homocysteine 濃度、提高體內氧化壓力及降低抗氧化能力。

在發炎反應過程中，血漿 PLP 是擔任細胞激素及其它多胜肽媒介物（polypeptide mediators）的輔酶 (Friso et al., 2001)。動物(Doke et al., 1998; Chiang et al., 2005)及人體(Roubenoff et al., 1995; Friso et al., 2001; Chiang et al., 2003; Friso et al., 2004; Kelly et al.,

2004; Morris et al., 2010; Sakakeeny et al., 2012)研究皆指出血漿 PLP 缺乏和增加前發炎細胞激素（如：TNF-α）及發炎指標[如：C-反應蛋白(C-reactive protein, CRP)、紅血球沉降係數(erythrocyte sedimentation rate, ESR)]濃度有顯著相關性。我們實驗室先前的研究(Huang et al., 2010)給予類風濕性關節炎病人高劑量的維生素 B-6 補充(100 mg PN/d)，發現可以降低前發炎細胞激素(如：IL-6 及 TNF-α)的濃度。肝硬化或肝癌患者因承受疾病壓力及處在發炎的狀況下，可能會增加體內對維生素 B-6 的代謝及利用，進而造成維生素 B-6 營養狀況缺乏。

維生素 B-6 被認為可保護細胞免於氧化壓力的威脅且其抗氧化能力甚至可能超過維生素 C 及 E (Bilski et al., 2000)。U937 單核細胞實驗指出維生素 B-6 可以避免因添加過氧化氫(H_2O_2)所生成的氧自由基及脂質過氧化(Kannan & Jain, 2004)。若添加維生素 B-6 於高同半胱胺酸血症的大鼠所食的葉酸缺乏但甲硫胺酸過多的的飲食，發現可降低血清 MDA 濃度(Mahfouz & Kummerow, 2004)。我們實驗室最近給予小鼠 homocysteine 誘發氧化壓力後，攝取維生素 B-6 缺乏小鼠的肝臟 MDA 濃度顯著高於維生素 B-6 攝取足夠及補充的小鼠(Hsu et al., 2015)。雖然無法確定維生素 B-6 的缺乏是否會直接降低清除自由基的能力，但有可能是透過其吡啶環上的氫氧基(－OH)及胺基(－NH_2)直接與過氧自由基結合進而清除自由基及降低脂質過氧化（Bilski et al., 2000; Kannan & Jain, 2004; Ohta & Foote, 2002）。

維生素 B-6 除了可能直接擔任抗氧化的功能外，也可以透過 GSH 抗氧化系統間接進行抗氧化功能。Homocysteine 經轉硫作用形成 cysteine 的過程中需要 PLP 作為輔酶，cysteine 與麩胺酸(glutamate)作用形成γ-glutamylcysteine，之後形成 GSH。GSH 在人體的解毒過程中扮演一個中心的角色。GSH 是穀胱甘肽硫轉移酶（glutathione *S*-transferase, GST）及 GPx 的重要輔因子。而這兩個抗氧化酵素的功能包括去除許多致腫瘤化合物的毒性及保護細胞免於氧化壓力的傷害（Hayes & McLellan, 1999）。在氧化還原過程中，GPx 將 H_2O_2 代謝成水的同時，也將 GSH 氧化成氧化型穀胱甘肽(oxidized glutathione, GSSH)，GSSH 再藉由穀胱甘肽還原酶(glutathione reductase, GR)將之還原成 GSH。另外，GST 會直接清除自由基，降低體內氧化壓力。因此，我們似乎可以合理的假設若肝臟功能受損導致 PLP 濃度不足或缺乏時可能會影響 cysteine 的生成，進而間接影響 GSH 的合成及整個 GSH 的抗氧化防禦系統的作用。但是有動物（Lima et al., 2006）及人體（Davis et al., 2006; Lamers et al., 2009）試驗指出即使維生素 B-6 攝取不足不僅不影響血漿 cysteine 濃度，還會增加血漿胱硫醚及 GSH 濃度；推測可能是因處在高氧化壓力或發炎狀態下，大量消耗體內貯存的維生素 B-6 而增加 GSH 的生成或從肝臟貯存釋放到血漿利用。我們也發現在給予小鼠 homocysteine 誘發氧化壓力後，維生素 B-6 缺乏組的小鼠其血漿 GSH 濃度顯著較對照組高但肝臟 GSH 濃度顯著較對照組低。值得注意的是血漿及肝臟 GSH 濃度的比值在維生素 B-6 缺乏組是 4.74%，對照組 2.78%及維生素 B-6 補充組 2.67% (Hsu et al., 2015)；我們的結果似乎支持在維生素 B-6 缺乏的狀態下，GSH 會從肝臟釋放到血漿中利用。雖然過去的研究指出肝硬化及肝癌患者的血漿 GSH 濃度低於健康受試者(Chawla et al., 1984; Bianchi et al., 1997)，肝臟組織 GSH 濃度顯著低於正常肝臟組織(Czeczot et al., 2006)，但是尚待釐清的是，肝硬化及肝癌患者的較低肝臟組織 GSH 濃度是因維生素 B-6 缺乏或不足時將 GSH 從肝臟釋放到血漿中利用，還是因其肝臟功能受損而直接造成的較低血漿及肝臟組織 GSH 濃度。維生素 B-6 及 GSH 在肝功能損傷、肝硬化及肝癌狀態下所扮演的角色尚待進一步釐清。

目的

動物試驗 – 以 BALB/c 小鼠為動物模式，利用低劑量乙醯胺酚 (acetaminophen, APAP) 逐漸誘發小鼠肝損傷，探討在小鼠逐漸形成肝功能壞死過程中：

1. 血漿及組織維生素 B-6 及 GSH 濃度與 homocysteine、cysteine、氧化壓力、GSSG 及 GSH 相關抗氧化酵素活性之相關性；
2. 單獨或合併給予維生素 B-6 及 GSH 的補充，血漿及組織維生素 B-6 及 GSH 濃度變化對 homocysteine、cysteine、氧化壓力、GSSG 及 GSH 相關抗氧化酵素活性之影響。

人體試驗 – 以健康受試者、肝硬化及肝硬化合併肝癌患者為研究對象，利用橫斷面 (cross section)、病例-對照(case-control)及介入(intervention)研究模式，探討：

1. 健康受試者、肝硬化及肝硬化合併肝癌患者的血漿維生素 B-6 及 GSH 濃度與發炎反應指標、homocysteine、cysteine、氧化壓力指標、GSSG 及 GSH 相關抗氧化酵素活性之差異性及關係；
2. 單獨或合併給予維生素 B-6 及 GSH 的補充對肝硬化及肝硬化合併肝癌患者的發炎反應指標、homocysteine、cysteine、氧化壓力指標、GSSG 及 GSH 相關抗氧化酵素活性之影響。

研究方法

<u>動物試驗</u>

實驗動物分組及飼養方法

40隻5週大雄性BALB/c小鼠購自財團法人國家實驗動物中心(台北、台灣)。小鼠飼養在中山醫學大學動物中心的不鏽鋼網籠，進食採自由攝食與飲水方式，飼料為顆粒 chew diet (AIN-93-M, MP Biomedicals, Inc., USA)，動物室環境溫度控制在25 ± 1°C，相對溼度為55 ± 5%，自動空氣調節 (換氣率每小時 12次)及光暗循環時間各為12小時。實驗動物的飼養管理及研究架構已通過中山醫學大學實驗動物照護及使用委員會 (IACUC Approval No. 1477)審查同意，並遵照科技部的實驗動物管理準則執行。

小鼠入室適應7天後，隨機分成5組。第一組為控制組，一星期3次以管灌給予0.9% 生理食鹽水 (10 μL/g BW)；第二組為乙醯胺酚[acetaminophen, *N*-acetyl-para-aminophenol, APAP)]組，以低劑量的APAP (400 mg/kg body weight) 溶於生理食鹽水中，一星期3次以管灌方式誘發小鼠肝損傷；第三組為B-6組，除了以低劑量的APAP (400 mg/kg body weight) 溶於生理食鹽水中，一星期3次以管灌方式誘發小鼠肝損傷外，此組小鼠每天攝取的是維生素B-6補充飼料(AIN-93-M飼料，添加14 mg/diet of PN-HCl, MP Biomedicals, Inc., USA)；第四組為GSH組，除了以低劑量的APAP (400 mg/kg body weight) 溶於生理食鹽水中，一星期3次以管灌方式誘發小鼠肝損傷外，此組小鼠每天攝取的是GSH補充飼料(AIN-93-M飼料，添加1 g/kg BW of GSH, MP Biomedicals, Inc., USA)；第五組則為B-6 + GSH組，除了以低劑量的APAP (400 mg/kg body weight) 溶於生理食鹽水中，一星期3次以管灌方式誘發小鼠肝損傷外，此組小鼠每天攝取的是維生素B-6及GSH補充飼料(AIN-93-M飼料，添加14 mg/diet of PN-HCl及1 g/kg BW of GSH, MP Biomedicals, Inc.,

USA)。之後於第4及8週，各組各犧牲4隻小鼠，取其肝組織。

生化分析

在試驗期間飲食和飲水自由攝取，且每週紀錄各組實驗動物攝食量和體重。小鼠犧牲後，取出肝臟組織秤重後進行分析。小鼠肝臟以100 mg之臟器加入1 mL磷酸鈉緩衝溶液 (PBS) (pH = 7.4) 之比例，於冰上均質後，以 12000 rpm、10分鐘、4℃離心，取上清液後，儲存於 -80°C冰箱直至分析。

維生素B-6

血漿、肝臟及腎臟的 PLP及PL以高效能液相層析儀 (high performance liquid chromatography, HPLC) 進行分析 (Talwar et al., 2003)。取 500 μL血漿或組織均質液，加入40 μL的 D.A. [semicarbazide (250 mg/dL)＋glycine (250 mg/dL)]，混合均勻後置於25℃恆溫水浴槽 30分鐘，加入 80 μL的過氯酸 ($HClO_4$) 後混合1分鐘，以高速離心機 (11000 rpm，4℃，10分鐘) 離心，取上清液 300 μL，加入 30 μL的 25 % NaOH，混合均勻後以過濾膜 (Filter : 0.45 μM，PVDF材質，Millipore 公司，美國) 過濾，取150 μL進行分析。使用之分離管柱為 Synergi 4u Fusion RP 80A (內徑5 μm，250 × 4.6 mm，Phrnomenx，美國)。移動相 (mobile phase) 包含 60 mmol/L的 NaH_2PO_4，內含 9.5 % methanol 及 400 mg/L的 EDTA，並以 85 %的 H_3PO_4調整 pH值至6.5。幫浦流速為 1.5 mL/min。螢光檢測器設定的 excitation波長為 380 nm， emission 波長為 450 nm。PLP的滯留時間約為7-8分鐘，PL的滯留時間約 28-29分鐘。以標準品的PLP及PL作出標準曲線，並進一步計算出檢體之濃度，單位以nmol/L表示。

同半胱胺酸與半胱胺酸

血漿及組織homocysteine與cysteine濃度的分析主要是參考 Dudman等人 (1996) 與 Radha Rama Devi等人(2006) 的方法，以 HPLC進行分析。取50 μL血漿或組織均質液，加入 100 μL的0.125 M borate buffer內含4 mM EDTA (pH = 9.22) 混合均勻後再加入 5 μL N-dimethyl-formamide內含 12.3 % tri-butylphosphine，混合均勻後於 4℃下，靜置 30分鐘。之後，加入150 μL 10 % trichloroacetic acid 混合均勻，離心 (11000 rpm，4℃，10分鐘)。再取 100 μL的上清液加入20 μL 1.5 M sodium hydroxide，混合均勻後於4℃下靜置10分鐘。之後，加入 250 μL 0.125 M borate buffer內含4 mM EDTA (pH = 9.22) 混合均勻後再加入100 μL fluorobenzo-2-oxa-1,3-diazole-4-sulfonic acid混合均勻後於 60℃下水浴1小時。之後，冷卻至室溫，以過濾膜 (Filter : 0.45 μM，PVDF材質，Millipore公司，美國) 過濾後取 150 μL進行分析。使用之分離管柱為 LiChrospher® 100 RP-18e (250 mm × 4 mm I.D，Merck，德國) ，guard column為LiChrospher® 100 RP-18e (5 μm，Merck，德國) 。移動相包括0.1 M potassium dihydrogen phosphate，內含 35 mL/L acetonitrile，並用 85 % phosphoric acid 調整 pH值至3.5。幫浦流速為1.2 mL/min。螢光檢測器設定的excitation 波長為385 nm，emission波長為515 nm。Cysteine滯留時間約為 3-4分鐘，Homocysteine滯留時間約為5-6 分鐘。以標準品的homocysteine及cysteine做出標準曲線，並進一步計算出檢體之濃度，單位以μmol/L表示。

硫代巴比妥酸反應物 (thiobarbituric acid reactive substances, TBARs)

此分析主要是參考 Lapenna等人(2001)的方法，利用低密度脂蛋白氧化過後次級產物MDA為指標，以螢光光譜儀測定反應物的產量，藉由反應物多寡判定MDA濃度，進而得知脂質過氧化的情形。取90 μL血漿或組織均質液，加入160 μL的phosphate buffered saline (PBS)混合均勻後加入250 μL的3% sodium dodecyl sulfate及1000 μL 0.1 N hydrochloride，混合均勻後加入150 μL 10 % phosphotungstic acid，混合均勻後再加入500 μL 0.7 % tribarbituric acid reactive substances，混合均勻後置於乾浴槽內，於100℃下反應30分鐘後，置於冷水中冷卻，最後加入2500 μL n-butanol萃取，混合均勻後以2000 rpm，10分鐘，24℃離心，並取上清液1000 μL，於螢光光譜儀設定excitation波長為515 nm，emission波長為555 nm下測得吸光值。因MDA無法由化學方法合成，因此用其類似物1,1,3,3-tetramethoxypropane取代以製做標準曲線，計算出TBARs反應物的濃度，單位以μmol/L表示。

蛋白質濃度

血漿、肝臟及腎臟組織蛋白質濃度的分析則是參考 Lowry 等人(1951)的方法。二價銅離子在鹼性溶液中會與蛋白質 peptide bonds 上的 nitrogen 結合，並還原為一價銅離子。一價銅離子與酪胺酸(***tyrosine)***、色胺酸(***tryptophan)***或 cysteine 使 Folin reagent 內的 phosphomolybdic-phosphotungstic acid (磷鉬酸與磷鎢酸) 還原為藍色。取 100 μL 稀釋之血漿或組織均質液，加入 100 μL 的 1 N NaOH，混合均勻後，加入 1 mL 的試劑 (含 2 % Na_2CO_3，0.5 % $CuSO_4$，1 % KNa tartrate，1N NaOH) 後，靜置 10 分鐘，再加入 100 μL 的 1 N Folin reagent，靜置 30 分鐘後，以分光光度計測量波長 750 nm 之吸光值。標準品以小牛血清蛋白 (bovine serum albumin, BSA) 製做標準曲線，並進一步計算檢體之蛋白質濃度，單位以 mg/mL 表示。

還原型及氧化型穀胱甘肽

血漿、肝臟及腎臟組織中的 GSH 及 GSSG 是以商業套組檢測 (glutathione assay kit, Cayman Chemical Company, Michigan, USA)。GSH 的檢測是藉由與 5,5－二硫二硝基苯甲酸 (5,5'-dithio-bis-2-nitrobenzoic acid, *DTNB) 結合之後會產生黃色產物 (5-thio*-2-nitrobenzoic acid, TNB)，及中間產物 GSTNB。GSTNB 會接著被 GR 還原成 TNB 及 GSH，TNB 增加的比例即可換算為樣本中 GSH 的濃度。利用 405 nm 的波長檢測 TNB 的吸光值，並以標準品曲線對照，換算出 GSH 的濃度。因 2-vinylpyridine 會抑制黃色產物 TNB 的產生，因此 GSSG 的測定是在分析 GSH 過程中添加 2-vinylpyridine 時測量。

穀胱甘肽還原酶活性

GR 活性是以商業套組檢測(glutathione reductase assay kit, Cayman Chemical Company, Michigan, USA)。GR 將 GSSG 還原成 GSH 的過程中同時將 NADPH 轉換成 $NADP^+$。因此 GR 的檢測是藉由 NADPH 氧化成 $NADP^+$時測量在波長 340 nm 下吸光值下降的速率。

人體試驗

研究架構

受試者

受試者募集

於臺中榮總一般外科及肝膽腸胃科募集肝硬化或肝硬化合併肝癌患者。納入條件為：1) 受試者須年滿 20 歲且小於 80 歲；且 2) 臨床、影像學檢查或病理切片組織經醫生診斷為肝硬化或肝硬化合併肝癌。病人若有以下條件將排除在本研究外：1) 目前正在服用任何營養素補充劑；2) 心臟、腎臟、腸胃道、糖尿病或其他代謝疾病；3) 服用干擾維生素 B-6 及 GSH 代謝的藥物〔例如：phenobarbital（酚巴比妥）, phenytoin（癲能停）、cycloserine（環絲胺酸）、pyrazinamide（比井醯胺）、isoniazid（異菸鹼醯肼）、(thio)semicarbazide（氨基鹽酸鹽）、hydramitrazine（美拉肼）、phenelzine（苯乙肼）、carbidopa（卡比多巴）、levodopa（左旋多巴）、hydralazine（阿普利素寧）、steroids（類固醇）、及 penicillamine（青黴胺）〕或葉酸阻抗性藥物〔如：sulfasalazine（斯樂腸溶錠）、methotrexate（甲氨喋呤）〕；或 4) 懷孕或哺乳。

於臺中榮總體檢科募集健康受試者。受試者納入條件為：1) 須年滿 20 歲且小於 80 歲；2) 無懷孕或哺乳中的婦女。若受試者有以下條件將排除在本研究外：1) 有心臟、肝臟、腎臟、腸胃道、糖尿病、酒精中毒、癌症或其他代謝疾病；2) 使用過荷爾蒙療法及服用任何會干擾維生素 B-6 及 GSH 代謝的藥物；3) 血液生化值異常，包括：尿素氮 > 24 mg/dL、肌酸酐 > 1.5 mg/dL、鹼性磷酸酶 > 130 IU/L、丙胺酸轉胺酶 > 40 IU/L、天門冬胺酸轉胺酶 > 40 IU/L、三酸甘油脂 > 200 mg/dL、總膽固醇 > 240 mg/dL、低密度脂蛋白 > 130 mg/dL 及高密度脂蛋白 < 35 mg/dL。

補充劑介入

肝硬化或肝硬化合併肝癌患者經簽屬知情同意書後被隨機分派至以下四組：1) 安慰組）；2) 維生素B-6組（50 mg/d）；3) GSH組（500 mg/d）；4) 維生素B-6 + GSH組 (50 mg/d維生素B-6 + 500 mg/d GSH)。受試者被要求每天早晚各補充一顆補充劑，介入3個月。補充劑的給予是以雙盲型式進行，因此安慰劑、維生素B-6及穀胱甘肽皆是以相同外觀的膠囊呈現，將膠囊分裝於藥袋內並編碼後交由研究人員轉交給受試者，無論是受試者或與受試者接觸的研究人員皆無法由外觀判斷所服用的究竟是維生素B-6、穀胱甘肽或安慰劑。補充劑介入將經臨床醫師評估同意後進行。安慰劑是購自新賀斯國際有限公司(New Health Products, Co., Ltd.)；維生素B-6補充劑是購自健安生技股份有限公司（Chin-Teng Pharmaceutical Industrial Co., Ltd., Taichung, Taiwan）;GSH補充劑 (KOHJIN Glutathione ®)是購自創百股份有限公司(Chambio Co., Ltd., Taichung, Taiwan)。

為了確定受試者服用補充劑的順從度，研究者每隔兩週提醒受試者服用補充劑。受試者每 1 個月須於約定之門診時間攜帶所剩餘的補充劑交由研究人員紀錄剩餘量，並計算其順從度。

資料收集

基本資料

基本資料包括年齡、性別、抽菸及酒精攝取習慣、家族病史及運動頻率。測量受試者的身高、體重、腰圍及臀圍，計算其身體質量指數 (body mass index, BMI; kg/m^2)。受試者休息至少五分鐘後測量血壓。

血液樣本

使用不含及含有抗凝血劑(EDTA 或 sodium citrate)之真空採血管 (Becton Dickinson, Rutherford, NJ) 採集每位參與橫斷面受試者的空腹血液，及另外採集參與介入研究的受試者在介入前、介入第 1、2 及第 3 個月的 16 mL 空腹血液。

生化分析

血液樣本進行下列各項生化分析：1) 臨床血液生化值；2) 維生素 B-6 營養狀況(血漿 PLP)；3) 發炎指標 [高敏感度 C-反應蛋白（high sensitivity CRP, hs-CRP）]；4) homocysteine；5) cysteine;6) GSH；9) GSSG；10) 氧化壓力指標(MDA 濃度)、11) GSH 相關抗氧化酵素活性(GPx、GR、GST)。血液樣本將會在抽血後一小時內進行離心（3000 rpm，10 分鐘），分離出上清液（血漿或血清）與血球。血液樣本儲存在-80°C 等待分析。

臨床血液生化值

臨床血液生化值包括白血球、總淋巴球計數、嗤中性球、血紅素、白蛋白、尿素氮、肌酸酐及 hs-CRP 是委託臺中榮民總醫院檢驗科代為分析。

維生素 B-6

血漿 PLP 以 HPLC 分析（Talwar et al, 2003）。螢光偵測器之 excitation 與 emission 波長分別為 320 及 420 nm。血漿 PLP、PL、4-PA 將會個別計算其組間變異數 (inter-assay) 及組內變異數 (intra-assay)。維生素 B-6 測定過程在微弱黃光下進行，避免光對維生素 B-6 的破壞。當血漿 PLP 濃度< 20 nmol/L 表體內維生素 B-6 缺乏 (Food and Nutrition Board, 1998; Leklem, 1990)，若血漿 PLP 濃度介於 20–30 nmol/L，則定義為臨界缺乏 (Leklem, 1990)。

同半胱胺酸及半胱胺酸

參考 Araki 及 Sako (1987) 的方法，利用 HPLC 分析。使用的分離管柱為 LiChrospher® 100 RP-18e (250 mm × 4 mm I.D，Merck)。移動相為 0.1 M KH_2PO_4 ，3.5% acetonitrile，pH = 3.5，流速為 1.2 mL/min，螢光檢測器之 excitation 波長為 385 nm，emission 波長為 515 nm。

氧化壓力指標

當自由基攻擊細胞膜的多元不飽和脂肪酸後會進行自由基的連鎖反應，導致體內脂質過氧化作用。參考 Jialal & Scaccini (1992) 的方法，取 90 μL 血漿加入 160 μL PBS 緩衝液，而後分別加入 0.1N HCl 及 10% 磷鎢酸 (phosphotungstic acid) 使蛋白質變性，之後加入 0.7 % TBA (thiobarbituric acid) 混合，於 100 °C 加熱 30 分鐘，待冷卻後，加入正丁醇，離心（2,000 rpm，10 分鐘），利用正丁醇萃取 TBA 與脂質過氧化產物-丙二醛（malondialdehyde, MDA）形成的紅色複合物質，測定螢光值 (excitation: 515 nm;

emission: 555 nm)。MDA 越多表示氧化程度越嚴重。

還原型及氧化型穀胱甘肽

GSH 及 GSSG 是以商業套組檢測 (glutathione assay kit, Cayman Chemical Company, Michigan, USA)。GSH 的檢測是藉由與 5,5－二硫二硝基苯甲酸 (5,5'-dithio-bis-2-nitrobenzoic acid, *DTNB) 結合之後會產生黃色產物 (5-thio*-2-nitrobenzoic acid, TNB)，及中間產物 GSTNB。GSTNB 會接著被 GR 還原成 TNB 及 GSH，TNB 增加的比例即可換算為樣本中 GSH 的濃度。利用 405 nm 的波長檢測 TNB 的吸光值，並以標準品曲線對照，換算出 GSH 的濃度。因 2-vinylpyridine 會抑制黃色產物 TNB 的產生，因此 GSSG 的測定是在分析 GSH 過程中添加 2-vinylpyridine 時測量。

GSH 相關抗氧化酵素活性

GPx 活性是利用商業套組檢測（glutathione peroxidase assay kit, Cayman Chemical Company, Michigan, USA）。GPx 可還原過氧化氫，降低 GSH 濃度及保護細胞免於氧化傷害。GPx 還原過氧化氫物時，會產生 GSSG，GSSG 則利用 GR 與 NADPH 將其還原回 GSH。而 NADPH 氧化成 $NADP^+$時會從 340 nm 的吸光值慢慢減少。在 340 nm 波長下吸光值減少的速率可作為 GPx 的活性。

GST 活性是以商業套組檢測(glutathione *S*-transferase assay kit, Cayman Chemical Company, Michigan, USA)。GST 可藉由將毒性物與穀胱甘肽結合以保護細胞。GST 的活性（細胞質及粒線體中）檢測是藉由 1-氯-2,4-二硝基苯和 GSH 的結合。結合時會從 340 nm 的吸光值慢慢增加。在 340 nm 波長下吸光值增加的速率可作為 GST 的活性。

GR 活性是以商業套組檢測(glutathione reductase assay kit, Cayman Chemical Company, Michigan, USA)。GR 將 GSSG 還原成 GSH 的過程中同時將 NADPH 轉換成 $NADP^+$。因此 GR 的檢測是藉由 NADPH 氧化成 $NADP^+$時測量在波長 340 nm 下吸光值下降的速率。

統計分析

資料將以 SAS 統計軟體 (version 9.13; The SAS Institute Inc., Cary, NC)進行分析。以 Kolmogorov-Smirnov test 分析資料是否成常態分布。動物試驗的同一組在第 4 及 8 週的生化數值是以 student *t*-test 或 Wilcoxon rank sum test 比較差異性(目的一)。五組在第 4 及 8 週的生化數值則是以 one way analysis of variance (ANOVA)或 ANOVA on ranks 比較差異性(目的二)。同組內不同時間點則是以 repeated measures of ANOVA 比較差異性。維生素 B-6 及 GSH 濃度與氧化壓力指標或 GSH 還原酶活性的關係是以 Pearson correlation coefficient 分析。統計結果是以 $p < 0.05$ 代表具有顯著差異或相關性。所有的數值以 means ± standard deviation (SD)呈現。

利用 Chi-square、one way analysis of variance (ANOVA)或 Krusakal-Wallis 比較各組間體位測量值及及生化檢測值之差異性。以 multiple linear regression analysis 調整干擾因子後，分析維生素 B-6 及 GSH 對 homocysteine、cysteine、發炎反應指標、氧化壓力程度及 GSH 相關抗氧化酵素活性的影響。以 two-way ANOVA 分析維生素 B-6 與 GSH 對罹患肝硬化或肝硬化合併肝癌間是否有交互作用。以 ANOVA 或是 Kruskal – Wallis one-way analysis on ranks 計算各組之間在第 0、1、2 及第 3 個月時各項數值的差異。one-way repeated measures ANOVA 或 Friedman repeated measures analysis of variance on ranks 比較各組內第 0、1、2 及第 3 個月時各項數值的差異。資料以 means ± SD 表示，$p < 0.05$ 具統計的意義。

結果

動物試驗

以 400 mg/kg body weight 的 APAP 持續每週管餵 3 次小鼠後，實驗過程中採自由進食和飲水，於第 4 週及第 8 週時每組各犧牲 4 之小鼠。第 4 週犧牲時的小鼠體重，在控制組、APAP、B6、GSH 以及 B6+GSH 組分別為 26.3 ± 0.3、26.5 ± 0.4、26.9 ± 0.4、25.6 ± 0.5、27.2 ± 0.5 公克。第 8 週犧牲時的小鼠體重，在控制組、APAP、B6、GSH 以及 B6+GSH 組分別為 28.1 ± 0.4、27.7 ± 0.3、27.2 ± 0.4、28.7 ± 0.5、28.2 ± 0.4 公克。五組小鼠的體重均無顯著差異。

第 4 週犧牲時的小鼠肝功能(ALT)，在控制組、APAP、B6、GSH 以及 B6+GSH 組分別為 40 ± 1.9、31.8 ± 1.4、30.5 ± 4.0、35.3 ± 3.8、29.5 ± 2.3 U/L。第 8 週犧牲時的小鼠肝功能(ALT)，在控制組、APAP、B6、GSH 以及 B6+GSH 組分別為 43 ± 3.7、45.5 ± 8.2、92.3 ± 32.6、60.0 ± 12.1、40.5 ± 3.5 公克 U/L，五組沒有顯著差異。

第 4 週及第 8 週小鼠的肝臟組織的維生素 B-6、同半胱胺酸、半胱胺酸、GSH、GSSG、GSH 還原酶活性、氧化壓力指標(丙二醛)以及總抗氧化能力的數值呈現在表一。五組在第 4 週時的各項數值皆無顯著差異。在第 8 週時除了丙二醛濃度在 B6+GSH 組較其他組別顯著增加外，其餘肝臟組織的各項數值無顯著變化。

人體試驗

共募集 110 位健康受試者，以及 75 位肝硬化或肝硬化合併肝癌患者。這部分的結果及討論因已發表在 Nutrients 期刊(Lai CY, Cheng SB, Lee TY, Liu HT, Huang SC, Huang YC*. Possible synergistic effects of glutathione and C-reactive protein in the progression of liver cirrhosis. Nutrients 2018;10:678; https://doi.org/10.3390/nu10060678)，故不再贅述，請見附錄一。

75 位肝硬化或肝硬化合併肝癌患者中，有 61 位患者同意參與介入研究，被隨機分派至以下四組：1) 安慰組，n = 14）；2) 維生素 B-6 組（50 mg/d，n = 14）；3) GSH 組（500 mg/d，n = 18）；4) 維生素 B-6 + GSH 組 (50 mg/d 維生素 B-6 + 500 mg/d GSH，n = 15) ，介入 3 個月。參與介入研究的受試者的基本資料呈現於 Table 2。四組受試者的年齡、BMI、收縮壓及舒張壓在第 0 個月(baseline)時皆無顯著差異。

受試者完成 3 個月的安慰劑、維生素 B-6、穀胱甘肽或是二者合併補充，其血漿各項生化值呈現於表 3。B-6 以及 B-6+GSH 組的受試者每天補充 50 mg 的維生素 B-6 後，其血漿 PLP 濃度較未補充前顯著上升。但是 GSH 及 B-6+GSH 組的受試者雖每天補充 500 mg 的 GSH，其血漿 GSH 濃度在整個補充劑介入期間並未有顯著變化。B-6 組的 GST 活性在第 12 週時較第 0 週時顯著增加，但 GPx 活性卻顯著下降。B6+GSH 組的總抗氧化能力在 12 週時較第 0 週顯著上升。

Table 1. Responses of biochemical measurements to placebo or supplementation in liver tissue of mice at week 4 and week 8

Parameters	**Week 4**				
	Control (n = 4)	**APAP (n = 4)**	**B-6 (n = 4)**	**GSH (n = 4)**	**B6 + GSH (n = 4)**
PLP (nmol/L)	349.29 ± 34.56	347.74 ± 15.63	365.42 ± 23.15	318.20 ± 15.39	379.18 ± 13.87
GSH (μmol/L)	3.83 ± 0.76	5.07 ± 1.66	4.47 ± 0.75	5.87 ± 1.60	7.40 ± 0.58
GSSG (μmol/L)	108.35 ± 10.81	104.86 ± 2.41	103.30 ± 10.81	93.87 ± 4.10	87.03 ± 11.51
GSSG/GSH ratio	33.28 ± 9.24	31.06 ± 11.04	25.69 ± 5.57	27.73 ± 14.72	12.02 ± 2.05
Homocysteine (μmol/L)	66.78 ± 6.21	67.14 ± 6.88	70.01 ± 5.27	75.33 ± 10.45	65.73 ± 3.68
Cysteine (μmol/L)	9.12 ± 1.00	9.87 ± 0.28	10.34 ± 0.82	8.92 ± 0.87	7.60 ± 0.36
MDA (μmol/L)	0.84 ± 0.22	0.46 ± 0.04	0.58 ± 0.05	0.78 ± 0.19	0.74 ± 0.06
GR (nmol/mL/min)	140.55 ± 14.53	151.41 ± 3.68	147.76 ± 14.74	128.73 ± 4.47	122.59 ± 9.56

Parameters	**Week 8**				
	Control (n = 4)	**APAP (n = 4)**	**B-6 (n = 4)**	**GSH (n = 4)**	**B6 + GSH (n = 4)**
PLP (nmol/L)	339.09 ± 19.02	300.34 ± 21.31	322.56 ± 10.55	325.56 ± 15.80	327.78 ± 23.77
GSH (μmol/L)	12.86 ± 2.29	7.95 ± 1.00	12.27 ± 2.22	11.73 ± 1.02	10.55 ± 1.13
GSSG (μmol/L)	123.65 ± 16.91	104.19 ± 3.40	123.31 ± 9.93	100.01 ± 4.63	101.62 ± 9.27
GSSG/GSH ratio	9.83 ± 0.67	13.67 ± 1.50	11.21 ± 2.44	8.68 ± 0.70	9.75 ± 0.57
Homocysteine (μmol/L)	78.53 ± 10.32	56.26 ± 5.5	74.36 ± 5.77	57.35 ± 5.79	63.66 ± 5.69
Cysteine (μmol/L)	9.17 ± 1.04	8.47 ± 0.24	9.18 ± 0.95	6.84 ± 0.32	7.27 ± 0.52
MDA (μmol/L)	0.49 ± 0.04^{b}	0.48 ± 0.01^{b}	$0.87 \pm 0.30^{a,b}$	$0.57 \pm 0.03^{a,b}$	0.82 ± 0.04^{a}
GR (nmol/mL/min)	127.30 ± 7.04	121.44 ± 4.32	127.88 ± 11.28	119.25 ± 3.55	122.52 ± 7.72

PLP, pyridoxal 5'-phosphate; GSH, glutathione; GSSG, oxidized glutathione; MDA, malondialdehyde; GR, glutathione reductase.

^{a,b,c} Values with different superscript letter are significantly different among groups, $p < 0.05$.

Table 2. Demographic and health characteristics of patients with liver cirrhosis[1]

	Placebo (n = 14)	Vitamin B-6 (n = 14)	GSH (n = 18)	B-6 + GSH (n = 15)
Age (y)	55.0 ± 3.4	62.9 ± 2.1	62.4 ± 2.3	56.4 ± 1.8
Sex (Male / Female)	10 / 4	9 /5	14 / 4	14 / 1
Body mass index (kg/m^2)	25.7 ± 1.2	26.0 ± 0.9	23.7 ± 0.9	25.4 ± 0.7
Blood pressure (mmHg)				
Systolic	136.7 ± 6.0	137.0 ± 6.0	127.6 ± 4.6	128.5 ± 3.3
Diastolic	83.1 ± 4.4	78.6 ± 3.6	79.4 ± 3.1	79.3 ± 2.3
Current smoking habits (n, %)	3, 21.4%	3, 21.4%	6, 33.3%	6, 40%
Current drinking habits (n, %)	1, 7.1%	2, 14.3%	1, 5.6%	426.7%

[1] Values are means ± standard error of mean (SE).

Table 3. Responses of biochemical measurements to placebo or supplementation in patients with liver cirrhosis[1]

	Placebo (n = 14)				B-6 (n = 14)			
Parameters	**wk 0**	**wk 4**	**wk 8**	**wk 12**	**wk 0**	**wk 4**	**wk 8**	**wk 12**
PLP (nmol/L)	67.6 ± 10.6	66.8 ± 19.0	71.2 ± 16.6	107.6 ± 35.7	55.2 ± 7.4^{b}	346.5 ± 78.4^{a}	359.1 ± 37.0^{a}	276.6 ± 38.5^{a}
GSH (μmol/L)	72.2 ± 7.0^{a,b}	59.8 ± 5.2^{b}	65.9 ± 8.1^{a,b}	79.6 ± 9.8^{a}	66.6 ± 10.0	59.7 ± 8.4	61.6 ± 7.6	68.6 ± 10.6
GSSG (μmol/L)	666.1 ± 18.5^{a,b}	626.9 ± 21.3^{b}	650.9 ± 15.6^{a,b}	694.2 ± 13.7^{a}	667.0 ± 18.3	651.2 ± 16.4	639.2 ± 10.8	679.3 ± 15.9
GSH/GSSG ratio	0.11 ± 0.01	0.10 ± 0.01	0.10 ± 0.01	0.11 ± 0.01	0.10 ± 0.01	0.09 ± 0.01	0.10 ± 0.01	0.10 ± 0.01
Homocysteine (μmol/L)	12.2 ± 2.4	10.6 ± 1.6	12.1 ± 1.8	12.5 ± 2.6	10.8 ± 0.7^{b}	11.4 ± 0.5^{a,b}	13.3 ± 0.9^{a}	12.9 ± 1.0^{a,b}
Cysteine (μmol/L)	202.5 ± 14.8	191.8 ± 7.0	199.9 ± 9.3	223.6 ± 11.1	217.2 ± 13.6	214.5 ± 16.7	208.5 ± 11.2	209.7 ± 10.6
MDA (μmol/L)	0.80 ± 0.05	0.81 ± 0.05	0.78 ± 0.04	0.87 ± 0.08	0.77 ± 0.04	0.71 ± 0.04	0.73 ± 0.04	0.80 ± 0.07
TEAC (μmol/L)	4335.3 ± 264.3	3986.1 ± 135.3	4147.6 ± 124.0	4412.1 ± 160.4	4448.8± 147.2^{a}	3961.6 ± 116.8^{b}	4164.1 ± 137.4^{a,b}	4321.3 ± 113.8^{a}
GST (nmol/mL/min)	116.2 ± 3.7	24.2 ± 4.2	17.6 ± 2.3	22.2 ± 4.5	16.3 ± 2.7^{b}	17.8 ± 3.0^{b}	12.7 ± 1.4^{b}	26.5 ± 3.3^{a}
GPx (nmol/mL/min)	199.4 ± 20.7	183.6 ± 17.8	174.1 ± 13.0	142.8 ± 10.8	211.9 ± 26.3^{a}	167.9 ± 16.5^{a,b}	179.6 ± 13.6^{a}	135.4 ± 8.1^{b}
GR (nmol/mL/min)	64.9 ± 4.1	68.6 ± 6.9	72.8 ± 6.2	68.3 ± 5.2	70.7 ± 4.0	77.1 ± 5.7	72.6 ± 4.4	72.7 ± 3.0
SOD (U/mL)	8.5 ± 0.9	7.8 ± 0.8	9.1 ± 1.0	7.8 ± 0.9	7.2 ± 1.0	7.7 ± 0.9	9.1 ± 0.9	8.4 ± 2.8
Catalase (nmol/mL/min)	85.7 ± 17.3	93.7 ± 12.5	64.5 ± 7.3	61.2 ± 10.1	62.9 ± 5.8	61.3 ± 5.0	60.7 ± 5.7	55.8 ± 8.5

	GSH (n = 18)				B-6 + GSH (n = 15)			
Parameters	**wk 0**	**wk 4**	**wk 8**	**wk 12**	**wk 0**	**wk 4**	**wk 8**	**wk 12**
PLP (nmol/L)	131.1 ± 33.8	97.7 ± 21.5	131.3 ± 41.0	63.9 ± 11.3	116.7 ± 37.1^{b}	389.6 ± 63.3^{a}	376.6 ± 63.5^{a}	319.9 ± 52.6^{a}
GSH (μmol/L)	77.4 ± 5.0	77.4 ± 12.7	90.8 ± 12.5	80.2 ± 5.8	78.1 ± 7.8	67.7 ± 9.5	68.1 ± 7.4	71.5 ± 7.2
GSSG (μmol/L)	673.1 ± 19.1	659.6 ± 15.0	676.9 ± 13.3	691.9 ± 16.2	662.6 ± 12.5^{a}	615.3 ± 17.7^{b}	664.8 ± 18.0^{a}	705.3 ± 17.3^{a}
GSH/GSSG ratio	0.12 ± 0.01	0.12 ± 0.02	0.13 ± 0.02	0.12 ± 0.01	0.12 ± 0.01	0.11 ± 0.01	0.10 ± 0.01	0.10 ± 0.01
Homocysteine (μmol/L)	10.3 ± 0.7	10.7 ± 0.7	11.8 ± 0.9	10.0 ± 0.5	9.9 ± 0.6	11.3 ± 1.1	10.1 ± 0.7	11.7 ± 1.1
Cysteine (μmol/L)	208.1 ± 9.8	208.6 ± 13.3	218.6 ± 10.0	225.7 ± 10.2	189.7 ± 9.3	196.4 ± 8.9	177.3 ± 9.7	192.2 ± 8.1
MDA (μmol/L)	0.78 ± 0.06	0.79 ± 0.06	0.78 ± 0.05	0.75 ± 0.05	0.81 ± 0.03	0.79 ± 0.05	0.78 ± 0.06	0.87 ± 0.06
TEAC (μmol/L)	4078.2 ± 112.4	4042.9 ± 97.4	4091.8 ± 114.8	4324.7 ± 97.0	3943.1 ± 143.7^{b}	4368.1 ± 104.9^{a}	3974.5 ± 119.0^{b}	4259.3 ± 103.2^{a,b}
GST (nmol/mL/min)	19.1 ± 3.4	20.1 ± 3.1	17.2 ± 2.0	27.0 ± 2.8	18.8 ± 2.6	16.0 ± 3.1	18.6 ± 1.7	25.6 ± 3.4
GPx (nmol/mL/min)	189.9 ± 12.6	213.2 ± 13.1	184.7 ± 12.7	168.7 ± 13.2	175.4 ± 16.4	162.7 ± 16.1	152.1 ± 9.9	151.3 ± 14.1

GR (nmol/mL/min)	85.0 ± 12.7	72.5 ± 5.3	75.1 ± 5.5	69.7 ± 4.0	80.3 ± 6.8	78.0 ± 7.2	75.0 ± 5.5	71.6 ± 26.2
SOD (U/mL)	7.1 ± 0.7	7.9 ± 0.8	8.6 ± 1.0	7.4 ± 0.7	8.2 ± 1.1	7.4 ± 0.9	9.1 ± 1.0	9.1 ± 0.7
Catalase (nmol/mL/min)	85.5 ± 13.5[a,c]	105.7 ± 18.1[a]	72.5 ± 8.5[a,c]	56.2 ± 5.5[c]	79.8 ± 8.7	85.0 ± 18.7	70.3 ± 8.9	71.5 ± 9.6

[1] Values are means ± standard error of mean (SE). PLP, pyridoxal 5'-phosphate; MDA, malondialdehyde; TEAC, trolox equivalent antioxidant capacity; SOD, superoxide dismutase; GSH, glutathione; GSSG, oxidized glutathione; GPx, glutathione peroxidase; GR, glutathione reductase; GST, glutathione *S*-transferase.

[a,b,c] Values with different superscript letter are significantly different within the group, $p < 0.05$.

討論

肝臟細胞功能異常或發生病變，可能會降低維生素 B-6 及 GSH 的代謝，進而影響人體重要的生理功能的進行。過去的研究已發現肝硬化及肝癌患者較健康受試者有較低的血漿 PLP (Mitchell et al., 1976; Labadarios et al., 1977; Anderson et al., 1980; Henderson et al., 1986; Zaman et al., 1986；Lin & Yin, 2007; Lin et al., 2011)及 GSH 濃度(Chawla et al., 1984; Bianchi et al., 1997)。較低的血漿 PLP 濃度可能不是因為患者攝取量不足或吸收較差而造成的，而是因為肝臟功能受損而降低維生素 B-6 磷酸化成 PLP 的效率以及增加 PLP 降解而造成的(Mitchell et al., 1976; Labadarios et al., 1977; Anderson et al., 1980; Zaman et al., 1986)。而較低的 GSH 濃度可能是肝硬化或肝癌患者因處於高度疾病壓力及發炎反應情況下，增加對 GSH 的利用及代謝。

過去研究給予肝硬化病人口服一次 25 mg PN 的補充，結果發現肝硬化病人口服 PN 後 2 小時可以增加 3 倍的血漿 PLP 濃度，且此濃度可以維持 24 小時 (Henderson et al., 1986)。若進一步給予肝硬化病人口服 28 天的 PN 補充(25 mg/d)，同樣可以改善肝硬化病人維生素 B-6 缺乏的情形 (Henderson et al., 1989)。本研究同樣發現給予 50 mg 維生素 B-6 補充後，肝硬化患者的血漿 PLP 濃度顯著上升。隨然維生素 B-6 補充可顯著增加患者血漿 PLP 濃度，但是血漿 PLP 濃度的增加並未反映在降低患者氧化壓力狀況及增加其抗氧化能力。可能的原因是患者未接受維生素 B-6 補充前的血漿 PLP 濃度並未成缺乏狀態(< 20 nmol/L)，充足的維生素 B-6 營養狀態應足以保護患者體內免於氧化壓力的威脅。

給予肝硬化受試者的 GSH 補充，卻未觀察到受試者的血漿 GSH 有顯著變化且未看到氧化壓力指標以及相關抗氧化酵素活性有顯著變化。過去研究也指出給予健康受試者每天口服 2 次 500 mg 的 GSH，4 週後顯示 GSH 的補充沒有顯著改變健康受試者的氧化壓力指標(尿液 F2-isoprostanes 及 8-hydroxy-2’-deoxyguanosine) (Allen & Bradley, 2011)。相反的，一個隨機、雙盲、安慰-控制的臨床試驗給予沒抽菸的健康受試者 250 mg 或 1000 mg 的口服 GSH 補充，結果顯示 GSH 補充後的 1、3 及 6 個月，受試者的血球 GSH 濃度顯著高於未補充前；攝取 1000 mg 的 GSH 的受試者在 6 個月後的紅血球、血漿及淋巴球濃度增加 30-35% (Richie et al., 2014)。似乎肝硬化患者無法有效利用 GSH 補充增加其身體儲存，因此也無法進一步降低氧化壓力及增加抗氧化能力。

綜合本研究結果，維生素 B-6 及 GSH 的補充無法降低肝損傷小鼠或肝硬化患者的氧化壓力，也無法增加其抗氧化能力。

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附錄一

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Possible Synergistic Effects of Glutathione and C-Reactive Protein in the Progression of Liver Cirrhosis

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Abstract: Liver cirrhosis is often associated with increased inflammatory responses and changes of glutathione (GSH) status. The possible interactions between these two factors in mediating damages of liver function remain unclear. Here, we measured the inflammatory responses and GSH status in liver cirrhotic patients and compared them with healthy subjects. In addition, we assessed the relationship of the GSH status and levels of inflammatory markers with the severity of the disease. This was a cross-sectional study. In total, we recruited 63 liver cirrhotic patients with Child–Turcotte–Pugh class A scores, and 12 patients with class B–C scores, together with 110 healthy subjects. Patients with class B–C scores showed the highest level of high-sensitivity C-reactive protein (hs-CRP) when compared with class A patients or healthy subjects. Patients in class A group had significantly higher GSH levels when compared with class B–C group or healthy subjects. After adjusting for potential confounders and each other, serum hs-CRP levels showed positive association with the Child–Turcotte–Pugh scores, while GSH levels showed negative association with Child–Turcotte–Pugh scores. Interactions were found between levels of plasma GSH and serum hs-CRP ($\beta = 0.004$, $p = 0.016$). CRP and GSH levels, which had showed interactions, were associated with the severity of liver cirrhosis.

Keywords: C-reactive protein; neutrophil-to-lymphocyte ratio; glutathione; Child–Turcotte–Pugh score; liver cirrhosis

1. Introduction

The end stage of liver fibrosis is liver cirrhosis, which involves the loss of liver cells and the formation of irreversible scarring. It is the 10th leading cause of death among adults in Taiwan (Health Promotion Administration, Ministry of Health and Welfare, Taiwan, 2016). The progression of liver cirrhosis is a multifactorial process, in which inflammation plays an important role. Such cirrhotic patients have elevated systemic inflammatory status [1–4]. Several markers of systemic inflammatory responses, such as C-reactive protein and neutrophil-to-lymphocyte ratio (NLR), are both inexpensive

and easy-to-measure systemic inflammatory markers, and likely to predict outcomes in cirrhotic patients [5–8].

During inflammation, the balance between pro- and antioxidants could be disrupted, leading to increased oxidative stress [4]. The results include damage to cellular components and altered gene expression, which further lead to the development or progression of liver cirrhosis [9–12]. In general, restoring the balance between oxidative stress and antioxidant defense capacities, or even tilting it towards a stronger antioxidant defense capacity, could protect the liver from further damage and slow down or limit the disease progression. Glutathione (GSH) is a thiol and tripeptide molecule synthesized in the liver. The molecule is involved in several reactions of detoxifying electrophiles and scavenging free radicals, as well as in suppressing hydrogen peroxide formation [13]. Since liver is responsible for ≥90% of GSH turnover and interorgan GSH homeostasis [14,15], liver cirrhosis could not only affect the endogenous production and utilization of GSH, but also cause the overconsumption of this antioxidant nutrient. Cirrhotic patients have lower levels of plasma or erythrocyte GSH compared with normal subjects [11,16–18], with whole blood GSH concentrations dropping and rising during different stages of liver cirrhosis [12], or with erythrocyte GSH levels increasing in parallel with the severity of liver cirrhosis [19]. The reports on GSH status in patients with different severities of liver cirrhosis are, however, inconsistent among investigators.

In spite of GSH being the most abundant cellular thiol antioxidant, GSH may also have an anti-inflammatory role [20,21]. Although both increased inflammatory responses and the changes of GSH status are very likely associated with liver cirrhosis, it is unclear whether their actions in mediating liver damage occur independently or interactively with each other. The purpose of this study was to characterize inflammatory responses and GSH status of patients in different stages of liver cirrhosis, and compare them with healthy subjects. In addition, we assessed whether GSH status and inflammatory markers were independent or interactive with each other to mediate the severity of liver cirrhosis.

2. Subjects and Methods

2.1. Study Design and Sample Size Calculation

This was a cross-sectional study. Galicia-Moreno et al. [12] reported a significant correlation of 0.59 between levels of whole blood GSH and Child–Turcotte–Pugh scores in alcoholic patients with liver cirrhosis. Based on that report, the sample size was estimated for a significant correlation of 0.35 between plasma GSH concentration and Child–Turcotte–Pugh scores in patients with liver cirrhosis at a power of 80% and a two-sided test with an α of 0.05. The minimal sample size required was 62 patients.

2.2. Subjects

Patients diagnosed with liver cirrhosis were recruited from the Division of General Surgery and Division of Gastroenterology and Hepatology of Taichung Veterans General Hospital, Taiwan. Diagnosis was made based on a variety of findings from clinical, histopathological, biochemical, and radiologic examinations. Biochemical parameters (i.e., serum albumin and bilirubin concentrations, prothrombin time), and subjective parameters (i.e., the degree of ascites and hepatic encephalopathy) were used to generate the score representing the severity of liver dysfunction. The severity scale is the following: class A (score 5–6), class B (score 7–9), and class C (score 10–15), which are derived from the Child–Turcotte–Pugh classification scheme [22]. Patients excluded were those with age ≤20 or ≥80 years, or with decompensated cirrhosis in clinical critical condition, pregnant or lactating, receiving chemotherapy, with diabetic, cardiovascular, renal, or chronic inflammatory diseases. Healthy subjects were recruited from the health management center of Taichung Veterans General Hospital, Taiwan. Subjects were excluded if they were ≤20 or ≥80 years of age, or had a history of chronic or metabolic

diseases. All subjects had given signed informed consent. This study was approved by the Institutional Review Board of Taichung Veterans General Hospital (IRB TCVGH No. SF14261B).

2.3. Data Collection and Biochemical Measurements

All personal data of subjects, like age, gender, and smoking and drinking habits, were first recorded. Their body height and weight were measured, and the body mass index (BMI) (kg/m^2) was calculated. Systolic and diastolic blood pressures were measured after a resting period of ≥5 min. Since silymarin has been shown to increase intracellular GSH level [23], we recorded whether patients were prescribed to take silymarin as supportive elements of liver cirrhosis.

On the appointment day, fasting blood samples were drawn and collected in vacutainer tubes (Becton Dickinson, Rutherford, NJ, USA) either with or without anticoagulant. Serum samples were used to measure, with an automated biochemical analyzer, the white blood cell count, absolute neutrophil count, absolute lymphocyte count, absolute monocyte count, albumin, total bilirubin, alanine aminotransferases (ALT), and creatinine. The NLR was calculated (absolute neutrophil counts divided by the absolute lymphocyte count). Estimated glomerular filtration rate was calculated based on the KDIGO CKD work group [24]. Serum high-sensitivity C-reactive protein (hs-CRP) concentrations were determined using a particle-enhanced immunonephelometer equipped with an image analyzer. Plasma samples of the patients were used to determine the following: levels of malondialdehyde (MDA), GSH, glutathione disulfide (GSSG, oxidized form of GSH), activities of glutathione peroxidase (GSH-Px) and glutathione reductase (GSH-Rd), and Trolox equivalent antioxidant capacity (TEAC). Plasma samples of healthy subjects were used to determine levels of MDA, GSH, GSH-Px activity, and TEAC. Plasma MDA levels that reflect oxidative stress were measured with thiobarbituric acid reactive substances excited at a wavelength of 515 nm and an emission wavelength of 555 nm, using a fluorescence spectrophotometer (Jasco FP-2020 plus, Hachioji, Tokyo, Japan) [25]. Plasma GSH and GSSG were measured with the GSH and GSSG commercial kits (BioVision Incorporate, Milpitas, CA, USA). Activities of plasma GSH-Px were determined with the GSH-Px commercial kit (Cayman Chemical Company, Ann Arbor, MI, USA). The plasma GSH-Rd activity was assessed according to the method of Carlberg & Mannervik [26]. TEAC was estimated according to the method of Erel [27]. All measurements were done in duplicates.

2.4. Statistical Analysis

Data analyses were done using the SAS statistical software package (version 9.3; Statistical Analysis System Institute Inc., Cary, NC, USA). The Shapiro–Wilk test was used for the normality test of sample distributions. Values presented in the text are means ± standard deviation. The median value and interquartile range were added in the parentheses for non-normally distributed values. Demographic characteristics and biochemical data were compared using a one-way analysis of variance or Kruskal–Wallis one-way analysis of variance of ranks, to determine significant differences across groups. Chi-square or Fisher's exact tests were used in the analyses of categorical variables. Multiple linear regression analyses, with the Child–Turcotte–Pugh score as a dependent variable, were used to assess the association of severity of liver cirrhosis with either individual or combined inflammatory indicators, GSH, GSSH, and GSH/GSSG ratio, after adjusting for potential confounders (i.e., age, gender, BMI, smoking and drinking habits, hepatitis, and silymarin use). Statistical significance was set at $p < 0.05$ (two-sided).

3. Results

Data from 75 patients with liver cirrhosis and 110 healthy subjects were analyzed. As the numbers of patients were too small in both class B and C (9 in class B, and 3 in class C), we chose to combine them into one group (class B–C) and the remaining patients (63) in another group (class A) for comparisons. There were 16 patients (25.4%) in class A group, and 3 patients (25%) in class B–C group were taking oral capsules of silymarin. Characteristics of patients and healthy subjects are shown in Table 1.

Patients in the class A group were older, had higher systolic blood pressure, serum ALT and creatinine levels compared with healthy subjects. Patients in class B–C group had the lowest serum albumin, and the highest serum total bilirubin levels, when compared to patients in class A group and healthy subjects. Table 2 shows the levels of inflammatory markers and indicators of oxidative stress and antioxidant capacity in patients and healthy subjects. Patients in group class B–C had the highest levels of hs-CRP compared with those of patients in class A and healthy subjects. Patients in class B–C had greater oxidative stress (i.e., in terms of plasma MDA levels) when compared to patients in class A and healthy subjects. Patients in class A had significantly higher levels of GSH, GSSG, GSH/GSSG ratio, and higher GSH-Px activity when compared to patients in class B–C or healthy subjects.

Table 3 shows the results from multiple linear regressions regarding the individual or combined effects of NLR, hs-CRP, GSH, GSSG, and GSH/GSSG ratio on the severity of liver cirrhosis. The NLR ratio and GSSG level were not associated with the Child–Turotte–Pugh scores after adjustment for potential confounders. Serum hs-CRP levels were significantly and positively associated with the scores, while GSH levels were significantly but negatively associated with the scores again after adjusting for basic potential confounders and each other. We found significant interactions between levels of serum hs-CRP and plasma GSH ($p = 0.016$), but we found no interactions between NLR and GSH. Results suggested that hs-CRP and GSH had interacted with each other in the association with Child–Turotte–Pugh scores.

Table 1. Demographic and biochemical characteristics of patients with liver cirrhosis and healthy subjects.

Characteristics	Liver Cirrhosis		Healthy Subjects ($n = 110$)
	Class A ($n = 63$)	Class B–C ($n = 12$)	
Age (y)	59.7 ± 9.3 [a]	57.6 ± 12.6 [a,b]	50.4 ± 7.9 [b]
Gender (Male/Female)	49/14	9/3	48/62
Height (cm)	162.5 ± 8.6 (163.5, 158.4–168.9)	167.7 ± 9.5	163.5 ± 7.9
Weight (kg)	65.9 ± 10.5 [a,b]	76.0 ± 15.1 [a]	62.9 ± 11.8 [b] (61.2, 53.6–71.3)
Body mass index (kg/m^2)	25.0 ± 3.6 [a]	27.1 ± 5.8 [a]	23.4 ± 3.4 [b] (22.8, 20.9–25.8)
Blood pressure (mmHg)			
Systolic	131.7 ± 18.1 [a]	127.3 ± 22.9 [a,b]	117.0 ± 17.6 [b]
Diastolic	79.4 ± 12.6	76.8 ± 15.6	75.0 ± 11.8
Serum albumin (g/dL)	4.2 ± 0.5 [b] (4.3, 3.9–4.6)	2.9 ± 0.4 [c]	4.5 ± 0.2 [a]
Serum creatinine (mg/dL)	1.0 ± 0.5 [a] (0.9, 0.8–1.0)	1.2 ± 0.7 [a] (0.9, 0.8–1.4)	0.8 ± 0.2 [b] (0.8, 0.6–0.9)
eGFR (mL/min/1.73 m^2)	81.5 ± 26.2 [b]	89.1 ± 47.7 [a,b]	98.0 ± 23.4 [a] (94.2, 80.4–112.2)
Serum ALT (U/L)	57.4 ± 59.0 [a] (38.0, 26–60.3)	43.9 ± 31.1 [a] (36.5, 23.0–53.0)	22.6 ± 7.4 [b] (22.0, 16.0–27.0)
Serum total bilirubin (mg/dL)	1.0 ± 0.6 [a] (0.9, 0.6–1.3)	4.5 ± 3.0 [b]	0.9 ± 0.5 [a] (0.9, 0.7–1.1)
Hepatitis (n, %)			
Hepatitis B	21, 33.3%	0	-
Hepatitis C	15, 23.8%	2, 16.7%	-
Co-hepatitis B and C	1, 1.6%	0	-
Current smoking habit (n, %)	17, 27.0%	6, 50%	17, 15.5%
Current drinking habit (n, %)	7, 11.1%	1, 8.3%	25, 22.7%

Values are means ± standard deviation. The median value and interquartile range are in the parentheses for non-normally distributed values. ALT, alanine aminotransferase; eGFR, estimated glomerular filtration rate. [a,b,c] Values with different superscript letter are significantly different among groups; $p < 0.05$.

Table 2. Inflammatory markers and oxidative stress indicators in patients with liver cirrhosis and healthy subjects.

Parameters	Liver Cirrhosis		Healthy Subjects (n = 110)
	Class A (n = 63)	Class B–C (n = 12)	
Inflammatory markers			
White blood cell (cells/mm^3)	5316.83 ± 2039.96 (5120.0, 3945.0–6550.0)	5216.00 ± 1953.31	5210.00 ± 1599.77 (5100.0, 4300.0–5700.0)
Absolute neutrophil count (cells/mm^3)	3410.07 ± 1556.4 (3012.68, 2289.6–4175.35)	3281.46 ± 1850.12 (3165.50, 2514.5–3411.0)	2971.63 ± 1170.50 (2823.45, 2332.0–3302.0)
Absolute lymphocyte count (cells/mm^3)	1413.51 ± 633.21	1315.45 ± 614.77	1705.98 ± 577.77 (1657.75, 1328.1–1947.0)
Neutrophil/Lymphocyte ratio	2.90 ± 1.75 ^{a} (2.35, 1.63–3.69)	2.50 ± 1.45 ^{a,b}	1.86 ± 0.85 ^{b} (1.69, 1.32–2.17)
Absolute monocyte count (cell/mm^3)	362.83 ± 170.03 (311.14, 239.12–471.3)	513.86 ± 255.30	376.53 ± 196.16 (340.4, 283.75–420.60)
hs-CRP (mg/dL)	0.99 ± 3.79 ^{b} (0.10, 0.04–0.26)	2.35 ± 4.77 ^{a} (0.75, 0.43–1.86)	0.06 ± 0.07 ^{c} (0.03, 0.01–0.07)
Indicators of oxidative stress and antioxidant capacity			
Malondialdehyde (μmol/L)	0.79 ± 0.19 ^{b} (0.76, 0.67–0.90)	0.93 ± 0.20 ^{a}	0.85 ± 0.19 ^{a,b} (0.81, 0.71–0.96)
Glutathione (μmol/L)	77.76 ± 25.41 ^{a}	42.88 ± 27.47 ^{b} (42.03,29.98–47.30)	44.69 ± 17.38 ^{b}
Glutathione disulfide (μmol/L)	667.03 ± 70.96 ^{a}	601.22 ± 87.64 ^{b}	-
GSH/GSSG ratio	0.12 ± 0.04 ^{a}	0.07 ± 0.04 ^{b}	-
GSH-Px (nmol/mL/min)	205.17 ± 68.33 ^{a} (206.30, 159.18–237.50)	183.80 ± 109.49 ^{a,b}	148.05 ± 36.57 ^{b} (140.08, 119.71–165.55)
GSH-Rd (nmol/mL/min)	76.73 ± 33.44 ^{b} (71.58, 61.84–83.58)	87.87 ± 23.51 ^{a} (83.59, 77.48–89.79)	-
TEAC (μmol/L)	4227.95 ± 640.79 (4188.40, 3886.14–4534.17)	4041.76 ± 819.51	4290.79 ± 468.35 (4290.93, 3974.34–4590.63)

Values are means ± standard deviation. The median value and interquartile range are in the parentheses for non-normally distributed values. hs-CRP, high sensitivity C-reactive protein; GSH, glutathione; GSSG, glutathione disulfide; TEAC, Trolox equivalent antioxidant capacity. ^{a,b,c} Values with different superscript letter are significantly different among groups; $p < 0.05$.

Table 3. Multiple linear regression analysis with Child–Turcotte–Pugh score as the dependent variable in patients with liver cirrhosis after adjusting for potential confounders.

Parameters	Covariate Model	β (Standard Error)	p Value
NLR	Confounders [1]	0.095 (0.084)	0.264
	GSH + confounders	0.101 (0.077)	0.197
	GSH + NLR × GSH + confounders	−0.264 (0.221)	0.237
hs-CRP	Confounders	0.074 (0.036)	0.068
	GSH + confounders	0.099 (0.035)	0.007
	GSH + hs-CRP × GSH + confounders	−0.275 (0.154)	0.079
GSH	Confounders	−0.014 (0.005)	0.007
	NLR + confounders	−0.016 (0.004)	<0.001
	hs-CRP + confounders	−0.017 (0.005)	<0.001
	NLR + NLR × GSH + confounders	−0.030 (0.009)	0.002
	hs-CRP + hs-CRP × GSH + confounders	−0.021 (0.005)	<0.001
NLR × GSH	NLR + GSH + confounders	0.005 (0.003)	0.084
hs-CRP × GSH	hs-CRP + GSH + confounders	0.004 (0.001)	0.016
GSSG	Confounders	−0.002 (0.002)	0.272
GSH/GSSG ratio	Confounders	−9.741 (3.468)	0.007

n = 75. β, regression coefficient. NLR, neutrophil-to-lymphocyte ratio; hs-CRP, high sensitivity C-reactive protein; GSH, glutathione; GSSG, glutathione disulfide. [1] Adjusted for age, sex, body mass index, smoking and drinking status, hepatitis, and silymarin use.

4. Discussion

Our findings indicated that inflammatory responses were elevated in patients with more severe liver cirrhosis. However, only CRP, but not NLR, was found here to be associated with Child–Turotte–Pugh scores. NLR may not faithfully reflect the inflammatory status of the liver, since lymphomononuclear cells are mainly involved in hepatic inflammation in those patients with chronic hepatitis and cirrhosis [28,29]. Tanoglu & Karagoz [30] have also cautioned that several clinical conditions may affect NLR levels. Therefore, in agreement with Tanoglu & Karagoz [30], the NLR level alone, without information from other contributing variables, might not provide accurate prognostic estimates of liver cirrhosis. On the other hand, while CRP is synthesized in the liver during acute phase of inflammation (as a response to cytokine productions), its concentration is not altered in impaired livers [31]. Since inflammatory responses and antioxidant capacities have not been examined in the previous studies regarding their possible interactions [5,7], the synergistic effects of CRP and GSH on the severity of liver cirrhosis cannot be ignored.

Although in the clinical setting, hepatic GSH concentration per se cannot be determined, plasma GSH concentration, which is measureable, could reflect, to some extent, its intrahepatic levels [32]. The ratio of reduced and oxidized forms of GSH (GSH/GSSG) in plasma is used to evaluate cellular oxidative stress [33], and the change from GSH to GSSG reduces cell proliferation and increases apoptosis [34]. We thus determined plasma GSH and GSSG concentrations, and calculate plasma GSH/GSSG ratio to represent intrahepatic levels and cellular oxidative stress, respectively. Previous studies indicated that plasma GSH concentration was similar, but plasma GSSG concentration was significantly higher in chronic hepatitis C patients with cirrhosis [17], and patients with alcoholic liver disease [35], when compared to the levels of healthy controls. Erythrocyte GSH concentrations are higher in class B cirrhotic patients than those in class A and healthy controls [19]. However, both the GSH concentration and GSH-Px activity, as found in the present study, were ~1.5 times higher in the class A group, and dropped to a level similar to that in the class B–C group, when compared to healthy subjects. This finding implied that patients with mild liver dysfunction increased their GSH turnover to cope with an increasing oxidative stress. Although we did not measure plasma GSH-Rd activity of healthy subjects and no previous published results can be compared, plasma GSH-Rd activity seemed to have an opposite performance with GSH-Px activity in class A and B–C group. It remains unclear whether GSH synthesis is unaltered or impaired with the further progression of liver cirrhosis. Among enzymes controlling for the utilization of GSH, γ-glutamyltransferase (also called γ-glutamyltranspeptidase, GGT) is a liver canalicular enzyme responsible for transporting GSH across cell membranes to provide the cells with the amino acids necessary for the de novo synthesis of GSH, and its defect might affect antioxidant defense, detoxification, and inflammation processes in the pathology of disease [36]. Since increased or decreased GGT activity has been shown to affect GSH utilization in patients with different kinds of liver diseases [36], we could not rule out the possibility that abnormal GGT activity might affect GSH utilization in our cirrhotic patients. However, abnormal GGT activity could be the functional result of liver cirrhosis, rather than the causal factor. Since GGT activity was not measured in the present study, the possible association of GGT activity with plasma GSH levels in cirrhotic patients requires further investigation.

The association between GSH status and cirrhotic severity has some pathogenic significances. However, their relationship remains controversial and unconfirmed. A previous study showed that whole blood GSH concentration is positively associated with Child–Pugh scores in patients with alcoholic liver cirrhosis [12]. In our study, however, plasma GSH concentration was negatively associated with the Child–Turcotte–Pugh scores. Another study also found that erythrocyte GSH concentration is negatively associated with the stages of hepatic fibrosis in patients with chronic hepatitis C [37]. The discrepancy in results across studies could be due to heterogeneity in the sampled patients, and different causative factors involved in cirrhosis. The inflammation that resulted from viral insult occurs mainly in the periportal hepatocytes (zone 1 of acinus), while that from toxins is in the perivenous hepatocytes (zone 3 of acinus) [38]. The intracellular GSH levels appear to vary across

different zones of hepatic acinus. The intracellular GSH concentration and the activity of synthesis of zone 1 hepatocytes are higher than those of zone 3 hepatocytes [39]. Since over half of our patients had chronic viral hepatitis, it is reasonable to speculate that the zone 1 hepatocytes, being injured more extensively, would be responsible for the lower GSH levels of class B–C cirrhotic patients. In agreement with previous studies [10,37], the gradual loss of GSH status in liver cirrhosis might be related to reduced hepatic GSH efflux or impaired GSH synthesis as the disease advances.

The uniqueness of this study is the simultaneous examination of the association of inflammatory responses and antioxidant capacity with the severity of liver cirrhosis. Four limitations of the study should be pointed out. The first is that the cases of liver cirrhosis were not evenly distributed across the severity scale. For example, due to the small numbers of patients, those in the Child–Turcotte–Pugh scores B and C had to be combined into a single group for analyses, and thus, scores, rather than classes, were practically used to determine the association of factors with the severity of the disease. Therefore, our results should be compared in more evenly distributed samples and with more patients recruited for analyses. The second is that all biochemical measurements were made at single time points. Results might not truly reflect the associations we were studying. Thirdly, GSSG levels and GSH-Rd activities were not measured in the healthy subjects due to limited volume of their blood samples we had obtained. Therefore, the picture of GSH metabolism could not be fully determined as we would have wished for better comparisons. Finally, a larger sample size may offer more statistical power to deal with the association between the severity of liver cirrhosis and inflammatory indicators and GSH status.

5. Conclusions

CRP and GSH had exerted synergistic effects in the association with the severity of liver cirrhosis. The decreased GSH antioxidant capacity and increased inflammatory responses should be considered by clinicians in the treatment of liver cirrhotic patients.

Author Contributions: C.-Y.L. and S.-B.C. who assisted with the study design, were in charge of the screening and recruitment of subjects and interpreting the results. T.-Y.L. and H.-T.L. recruited subjects and critically revised the manuscript. S.-C.H. analyzed biochemical measurements and performed data coding. Y.-C.H. was responsible for the study design, interpretation of results, and preparation of the manuscript. All authors read and approved the final revision of the manuscript.

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Conflicts of Interest: The authors declare no conflict of interest. However, part of results is presented in the conference PENSA, 2018 (Seoul, Korea). This study is part of Chia-Yu Lai (first author) doctoral research.

Abbreviations

The following abbreviations are used in this manuscript:

ALT	alanine aminotransferases
BMI	body mass index
hs-CRP	high-sensitivity C-reactive protein
GSH	glutathione
GSH-Px	glutathione peroxidase
GSH-Rd	glutathione reductase
GSSG	oxidized glutathione
MDA	malondialdehyde
NLR	neutrophil-to-lymphocyte ratio
TEAC	Trolox equivalent antioxidant capacity

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科技部補助專題研究計畫出席國際學術會議心得報告

日期：2018 年 6 月 30 日

計畫編號	MOST　104－2320－B－040－009　－　MY3		
計畫名稱	維生素 B-6 與穀胱甘肽的單獨及協同作用對肝功能損傷小鼠以及肝硬化及肝硬化合併肝癌患者的發炎反應、同半胱胺酸代謝、氧化壓力及抗氧化能力的影響 (第三年)		
出國人員姓名	黃怡嘉 / 葉恩菱	服務機構及職稱	中山醫學大學營養系教授 / 營養系博士班候選人
會議時間	2018 年 6 月 13 日 至 2018 年 6 月 16 日	會議地點	韓國首爾
會議名稱	The 19th Congress of PENSA (Parenteral and Enteral Nutrition Society of Asia) 2018		
發表題目	Glutathione and C-reactive protein are associated with the severity of liver cirrhosis (poster)		

一、參加會議經過

PENSA 是由亞洲國家輪流舉辦的學術暨臨床營養會議，2018 年的 PENSA 會議於 6 月 13－6 月 16 日在韓國首爾召開。此次因獲得科技部專題研究計畫（MOST　104－2320－B－040－009－MY3）補助出席國際學術會議，計畫主持人和博士班學生(葉恩菱)榮幸的能參與此次的國際研討會（圖一），與營養及醫學等相關領域學者齊聚一堂，分享彼此研究心得及聆聽大會邀請的國際著名講者精湛的演說。

PENSA 會議議程(表一)是由不同臨床營養與醫學的 session 所組成，各個 session 邀請在其臨床營養領域中的醫師、營養師以及營養學者做其專題演講，內容包括：NST Quality assurance and accreditation, Optimizing nutrition in sarcopenia 以及 Glutamine supplements in critically ill patients, Mitochondria and nutrition, Cancer and nutrition, Medical foods in the world, Nutrition in obesity, Clinical nutrition research, Intestinal failure, Comprehensive nutritional approach after pancreatectomy, Microbiota for health & nutrition, Nutritional Screening/Assessment: Does One Fit All?, How to Reduce Artificial Nutrition Related Complications: Data-driven Approach, Micronutrients, Liver transplantation section, 以及 Hot nutrition topics in critically ill patients 等。每個 session 的演講內容都是根據主題所延伸出來的相關副題。除了演講外，也有全天候的 posters 展示。大會對其內容的安排非常多元、緊湊且充實，讓參與者有如置身學術及臨床研究的殿堂，透過聆聽演講與其他研究者的心得交流，讓計畫主持人獲益良多。

此次除了參加每日的演講外，也以壁報的形式（poster presentation）(圖一)發表研究成果，發

表的作者及標題分別為：Huang YC, Lai CY, Cheng SB, Liu HT. Glutathione and C-reactive protein are associated with the severity of liver cirrhosis。在壁報展示的過程中有許多位的國外學者提出他們對我們的研究結果的問題及見解，並展開熱烈的討論，事後並且互留聯絡方式，期待將來也許有國際合作的機會。

二、與會心得

PENSA 是由亞洲國家的各腸道靜脈營養學會輪流在不同城市所舉行的國際聯合臨床營養會議，是一個大型且多元的臨床營養及醫學研討會。此次能獲得科技部專題計畫出席國際會議的差旅費用的補助，讓計畫主持人可以至韓國首爾參與此次的學術盛會，榮幸能與營養與醫學等相關領域學者齊聚一堂，共同聆聽台上講者精湛的演講並參與討論，不僅增加與國外學術研究學者的交流，也開拓對自己的研究深度及廣度，真是不虛此行。

三、發表壁報

中山醫學大學 Chung Shan Medical University 誠・愛・精・勤

Abstract Number: ABST-0100

Glutathione and C-reactive protein are associated with the severity of liver cirrhosis

Yi-Chia Huang[1], Chia-Yu Lai[2], Shao-Bin Cheng[2,3], Hsiao-Tien Liu[2,3]

[1]Department of Nutrition, Chung Shan Medical University; [2]Division of General Surgery, Department of Surgery, Taichung Veterans General Hospital; [3]School of Medicine, Chung Shan Medical University, Taiwan.

Abstract

Although increased inflammatory responses and the change of glutathione (GSH) status might be associated with liver cirrhosis, it is unclear whether they act independently or interact with each other to mediate the damage of liver function. The purpose of this study was to assess whether GSH status and inflammatory markers had interaction with each other to be associated with the severity of liver cirrhosis. Sixty-three liver cirrhotic patients with Child-Turcotte-Pugh class A, 9 patients with class B and 3 patients with class C, and 110 healthy subjects were recruited from the Division of General Surgery and Division of Gastroenterology and Hepatology of Taichung Veterans General Hospital, Taiwan. Patients with class B-C score had the highest high-sensitivity C-reactive protein (hs-CRP) concentration than those of patients with class A and healthy subjects. Patients with class A had significantly higher GSH concentration when compared to patients with class B-C or healthy subjects. Serum hs-CRP concentration was significantly positively associated with the Child-Turcotte-Pugh score, while GSH concentration was significantly negatively associated with Child-Turcotte-Pugh score after adjusting for potential confounders. There was an interaction between plasma GSH and serum hs-CRP levels ($p = 0.03$). Both CRP and GSH had significant roles and interact with each other to be associated with the severity of liver cirrhosis. This study was supported by the Ministry of Science and Technology, Taiwan (MOST 104-2320-B-040-009-MY3) and Taichung Veterans General Hospital (TCVGH-1074601C).

Introduction

The end stage of liver fibrosis is liver cirrhosis which involves the loss of liver cells and the formation of irreversible scarring. It is the 10th cause of death among adults in Taiwan (Health Promotion Administration, Ministry of Health and Welfare, Taiwan, 2016). The progression of liver cirrhosis is a multifactorial process, in which inflammation plays an important role. During inflammation, the balance between pro- and anti-oxidants could be disrupted, leading to increased oxidative stress. In general, restoring the balance between oxidative stress and antioxidant defense capacities, or even tilted it towards a stronger antioxidant defense capacity, could protect liver from further damages and slow down or limit the disease progression. Glutathione (GSH) is involved in several reactions of detoxifying electrophiles and scavenging free radicals, as well as in suppressing hydrogen peroxide formation. Since liver is responsible for ≥ 90% of GSH turnover and interorgan GSH homeostasis, liver cirrhosis could affect the endogenous production and utilization of GSH. However, the reports on GSH status in patients with different severities of liver cirrhosis are however inconsistent among investigators. Although both increased inflammatory responses and the changes of GSH status are very likely associated with liver cirrhosis, it is unclear whether their actions in mediating liver damages occur independently or interactively with each other.

Purpose

The purpose of this study was to assess whether GSH status and inflammatory markers had interaction with each other to be associated with the severity of liver cirrhosis.

Subjects & Methods

Patients with liver cirrhosis were recruited from the division of general surgery and division of gastroenterology and hepatology of Taichung Veterans General Hospital, Taiwan. The severity of liver dysfunction was assessed based on the Child-Pugh classification. Patients were excluded if they were younger than 20 y or older than 80 y, were pregnant or lactating, were receiving chemotherapy, or had diabetes, cardiovascular or renal diseases. Informed consent was signed by each participated patient. Control subjects were recruited from the health management center of Taichung Veterans General Hospital, Taiwan. Control subjects were excluded if they were younger than 20 y or older than 80 y, had history of chronic or metabolic diseases. This study was approved by the Institutional Review Board of Taichung Veterans General Hospital.

Serum samples were used to measure white blood cell count, neutrophil count, lymphocyte count, albumin, alanine aminotransferases, and creatinine. The neutrophil to lymphocyte ratio (NLR) was calculated (absolute neutrophil counts divided by the absolute lymphocyte count). Serum high-sensitivity C-reactive protein (hs-CRP), GSH, and glutathione disulfide (GSSG, oxidized form of GSH) concentrations were determined.

Data analyzes were done using the SAS statistical software package (version 9.3; Statistical Analysis System Institute Inc., Cary, NC, USA). The Shapiro-Wilk test was used for the normality test of sample distributions. One-way analysis of variance or Kruskal-Wallis one way analysis of variance of ranks were used to determine significant differences across groups. Chi-square or Fisher's exact tests were used in the analyses of categorical variables. Multiple linear regression analyses, with the Child-Turcotte-Pugh score as dependent variable, were used to assess the association of severity of liver cirrhosis with either individual or combined inflammatory indicators, GSH, GSSH and GSH/GSSG ratio after adjusting for potential confounders. Statistical significance was set at $p \leq 0.05$ (two-sided).

Results

There were 75 patients with liver cirrhosis and 110 healthy subjects in this study. We combined class B and C (9 in class B, and 3 in class C) into one group (class B-C) and the remaining patients (63) in another group (class A) for comparisons. Patients in the class A group were older, had higher systolic blood pressure, serum ALT and creatinine levels compared with healthy subjects. Patients in class B-C group had the lowest serum albumin levels when compared to patients in class A group and healthy subjects.

Table 1. Inflammatory markers, glutathione and glutathione disulfide in patients with liver cirrhosis and healthy subjects

	Liver cirrhosis		Healthy subjects (n = 110)
	Class A (n = 63)	Class B-C (n = 12)	
White blood cell (/mm³)	5316.83 ± 2039.96	5216.00 ± 1953.31	5210.00 ± 1599.77
Neutrophil (% of WBC)	63.00 ± 10.01[a]	60.68 ± 13.96[a,b]	56.25 ± 8.44[b]
Lymphocyte (% of WBC)	26.90 ± 9.28[a]	28.24 ± 10.87[a,b]	33.55 ± 7.69[b]
NLR	2.90 ± 1.75[a]	2.50 ± 1.45[a,b]	1.86 ± 0.85[b]
hs-CRP (mg/dL)	0.99 ± 3.79[b]	2.35 ± 4.77[a]	0.06 ± 0.07[c]
GSH (μmol/L)	77.76 ± 25.41[a]	42.88 ± 27.47[b]	44.69 ± 17.38[b]
GSSG (μmol/L)	667.03 ± 70.96[a]	601.22 ± 87.64[b]	–
GSH / GSSG ratio	0.12 ± 0.04[a]	0.07 ± 0.04[b]	–

Values are means ± standard deviation with the median value in the parentheses. WBC, white blood cell; NLR, neutrophil/lymphocyte ratio; hs-CRP, high sensitivity C-reactive protein; GSH, glutathione; GSSG, glutathione disulfide. [a,b,c]Values with different superscript letter are significantly different among groups; $p < 0.05$.

Table 2. Multiple linear regression analysis with Child- Turcotte-Pugh score as the dependent variable in patients with liver cirrhosis after adjusting for potential confounders

	Child- Turcotte-Pugh score	
Covariate model	β (standard error)	p value
Confounders + NLR	0.134 (0.083)	0.112
+ GSH	-0.014 (0.005)	0.005
Confounders + NLR	-0.190 (0.237)	0.426
+ GSH	-0.026 (0.010)	0.008
+ NLR × GSH	0.004 (0.003)	0.151
Confounders + hs-CRP	0.097 (0.037)	0.011
+ GSH	-0.014 (0.005)	0.010
Confounders + hs-CRP	-0.265 (0.167)	0.117
+ GSH	-0.017 (0.005)	0.002
+ hs-CRP × GSH	0.004 (0.002)	0.030

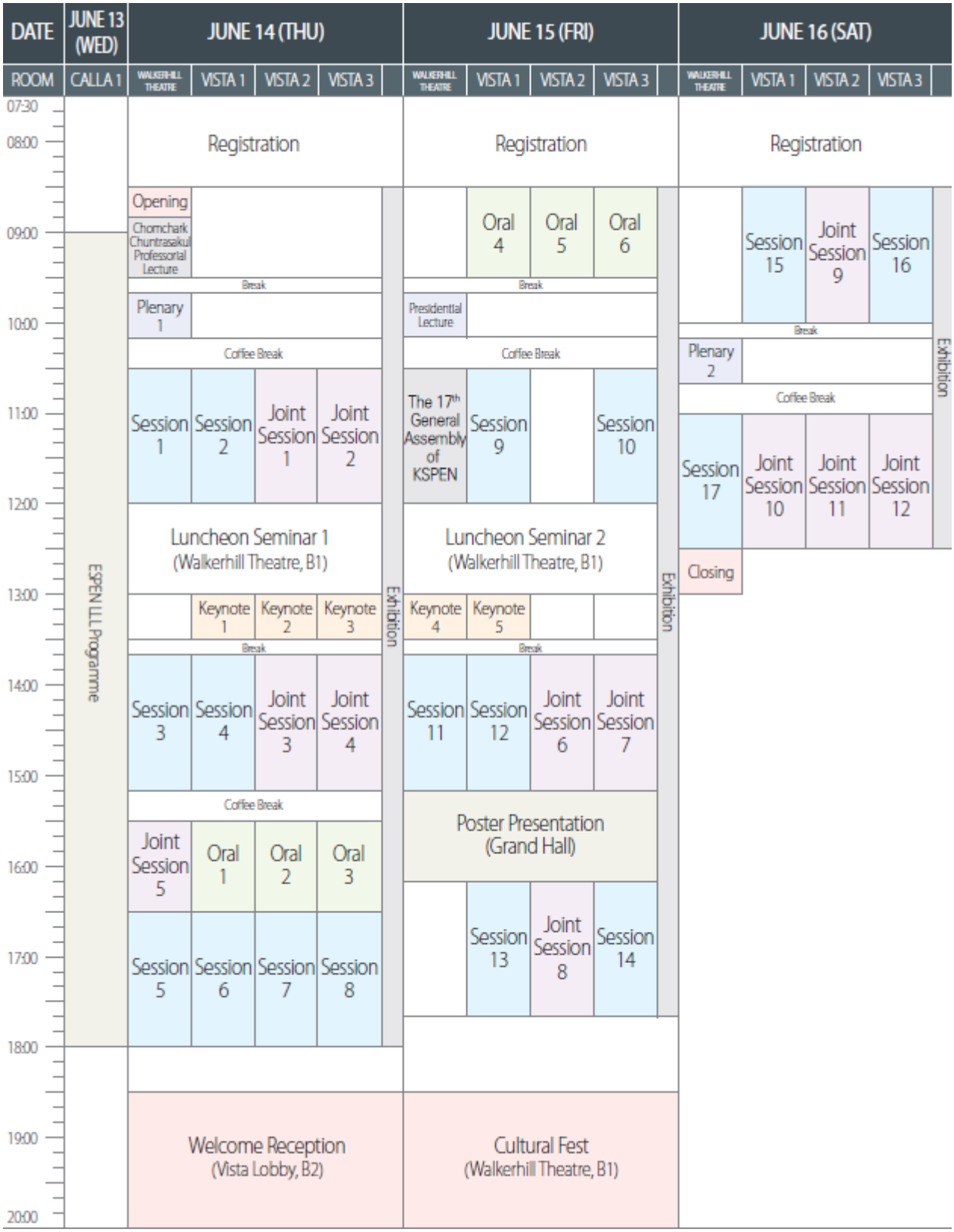
n = 75. β, regression coefficient. NLR, neutrophil to lymphocyte ratio; hs-CRP, high sensitivity C-reactive protein; GSH, glutathione. Adjusted for age, sex, body mass index, smoking and drinking status.

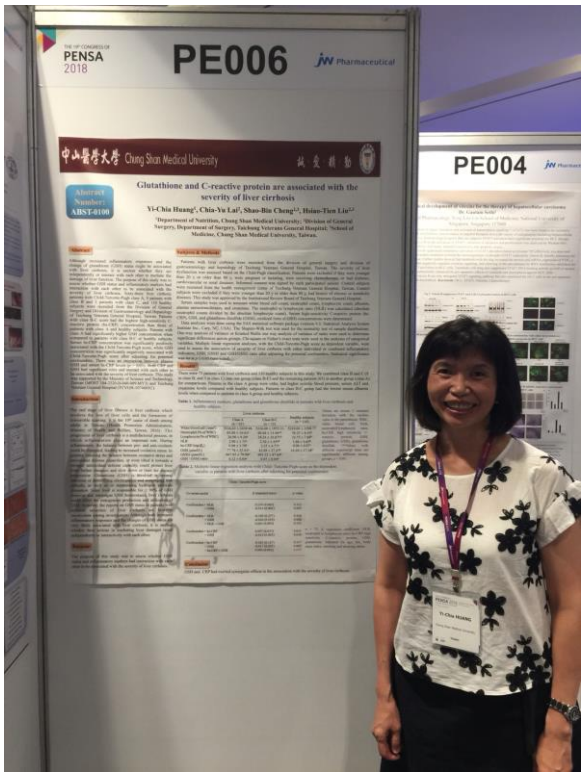
Conclusion

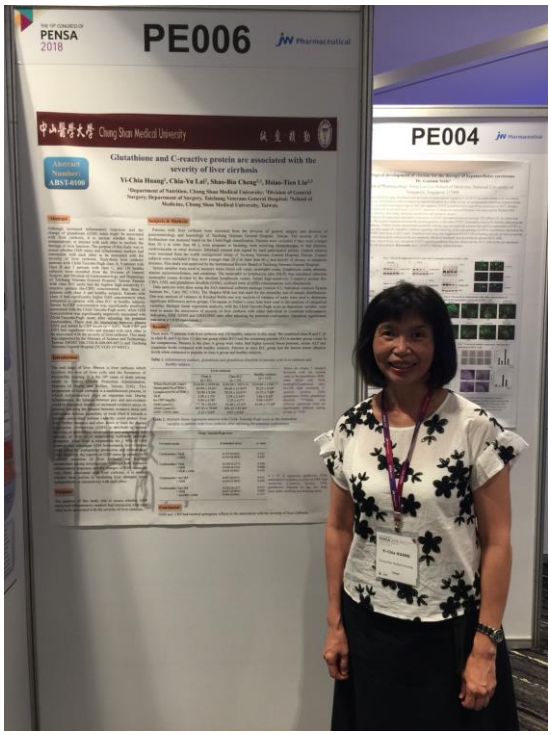
GSH and CRP had exerted synergistic effects in the association with the severity of liver cirrhosis.

四、攜回資料名稱及內容

表一：大會議程







圖一：研討會會場及壁報發表

五、其他

1. 研討會邀請函

19th CONGRESS OF
PENSA 2018
Innovative Nutrition in Global Health

Parenteral and Enteral Nutrition Society of Asia
in conjunction with The 17th Annual Congress of KSPEN
JUNE 13 - 16, 2018 SEOUL, KOREA

May 21, 2018

Name: Yi-Chia HUANG
Affiliation: Chung Shan Medical University
Country: Taiwan

Dear Dr. Yi-Chia HUANG,

On behalf of the 19th Congress of PENSA (Parenteral and Enteral Nutrition Society of Asia) Organizing Committee, we wish to extend to you an invitation to travel to Seoul, Korea to attend PENSA 2018, which will take place from June 13th to 16th, 2018, at Grand Walkerhill Seoul.

Under the theme of 'Innovative Nutrition in Global Health,' professionals from the field of Parenteral and Enteral Nutrition will gather together with the goal of the further development and future success of related fields. We plan to offer special events as well as the most authoritative and stimulating scientific program. In addition, participants will have a great networking opportunity through diverse social activities.

For more details regarding the congress, please visit our website (http://www.pensa2018.org) or contact the secretariat (seoul@pensa2018.org).

We do hope we shall have the pleasure of welcoming you to the congress this coming June.

Best wishes,

Ho-Seong Han

Ho-Seong Han, MD, PhD
President of PENSA 2018

※This invitation letter is an official document for the purpose of securing travel permission from your institution or the Korean embassy. Be advised, this invitation does not mean the organizing committee will cover your registration fee and travel expenses. You will be responsible for the registration fee and your own travel expenses that include airfare, ground transportation, hotel, meals and travel insurance.

2. 研討會註冊證明

19th CONGRESS OF
PENSA 2018
Innovative Nutrition in Global Health

Parenteral and Enteral Nutrition Society of Asia
in conjunction with The 17th Annual Congress of KSPEN
JUNE 13 - 16, 2018 SEOUL, KOREA

RECEIPT

On behalf of the Organizing Committee of
the 19th Congress of PENSA (Parenteral and Enteral Nutrition Society o
f Asia),
we appreciate your registration.
We have duly received the amount below.

Name	Yi-Chia HUANG
Affiliation	Chung Shan Medical University
Country	Taiwan
Category	Non Physicians
Registration Fee	**USD** 250
Method of Payment	Card
Date of issue	2018-03-22
Total Amount	**USD** 250

Ho-Seong Han

HO-SEONG HAN, MD, PHD
PRESIDENT OF PENSA 2018

104年度專題研究計畫成果彙整表

計畫主持人：黃怡嘉	**計畫編號：**104-2320-B-040-009-MY3
計畫名稱：維生素B-6與穀胱甘肽的單獨及協同作用對肝功能損傷小鼠以及肝硬化及肝硬化合併肝癌患者的發炎反應、同半胱胺酸代謝、氧化壓力及抗氧化能力的影響	

成果項目					量化	單位	質化 （說明：各成果項目請附佐證資料或細項說明，如期刊名稱、年份、卷期、起訖頁數、證號...等）
國內	學術性論文	期刊論文			0	篇	
		研討會論文			0		
		專書			0	本	
		專書論文			0	章	
		技術報告			0	篇	
		其他			0	篇	
	智慧財產權及成果	專利權	發明專利	申請中	0	件	
				已獲得	0		
			新型/設計專利		0		
		商標權			0		
		營業秘密			0		
		積體電路電路布局權			0		
		著作權			0		
		品種權			0		
		其他			0		
	技術移轉	件數			0	件	
		收入			0	千元	
國外	學術性論文	期刊論文			1	篇	Lai CY, Cheng SB, Lee TY, Liu HT, Huang SC, Huang YC*. Possible synergistic effects of glutathione and C-reactive protein in the progression of liver cirrhosis. Nutrients 2018;10:678; https://doi.org/10.3390/nu1006067
		研討會論文			2		1. Huang YC, Cheng SB, Liu HT, Lai CY. Glutathione concentration and glutathione peroxidase activity are associated with the risk of liver cirrhosis independently of oxidative stress. Experimental Biology 2017. FASEB J 2017 (poster) 2. Huang YC, Lai CY, Cheng SB, Liu HT. Glutathione and C-reactive protein are associated with the severity of liver cirrhosis. PENSA 2018 (poster)

		專書			0	本	
		專書論文			0	章	
		技術報告			0	篇	
		其他			0	篇	
	智慧財產權及成果	專利權	發明專利	申請中	0	件	
				已獲得	0		
			新型/設計專利		0		
		商標權			0		
		營業秘密			0		
		積體電路電路布局權			0		
		著作權			0		
		品種權			0		
		其他			0		
	技術移轉	件數			0	件	
		收入			0	千元	
參與計畫人力	本國籍	大專生			0	人次	
		碩士生			6		此3年期計畫共有6位碩士生，陳心爰、彭逸珊、張藝馨、鍾佩珊、蕭詠方以及陸晴參與研究。
		博士生			1		此3年期計畫共有1位博士生，葉恩菱參與研究。
		博士後研究員			0		
		專任助理			0		
	非本國籍	大專生			0		
		碩士生			0		
		博士生			0		
		博士後研究員			0		
		專任助理			0		

其他成果 （無法以量化表達之成果如辦理學術活動、獲得獎項、重要國際合作、研究成果國際影響力及其他協助產業技術發展之具體效益事項等，請以文字敘述填列。）	Experimental Biology 2016（美國聖地牙哥）研討會，American Society for Nutrition邀請計畫主持人在The International Forum - Taiwan演講，講題為「Vitamin B-6 and Homocysteine with Inflammation and Oxidative Stress in Certain Diseases」

科技部補助專題研究計畫成果自評表

請就研究內容與原計畫相符程度、達成預期目標情況、研究成果之學術或應用價值（簡要敘述成果所代表之意義、價值、影響或進一步發展之可能性）、是否適合在學術期刊發表或申請專利、主要發現（簡要敘述成果是否具有政策應用參考價值及具影響公共利益之重大發現）或其他有關價值等，作一綜合評估。

1. 請就研究內容與原計畫相符程度、達成預期目標情況作一綜合評估
   ■達成目標
   □未達成目標（請說明，以100字為限）
   　□實驗失敗
   　□因故實驗中斷
   　□其他原因
   說明：

2. 研究成果在學術期刊發表或申請專利等情形（請於其他欄註明專利及技轉之證號、合約、申請及洽談等詳細資訊）
   論文：■已發表　□未發表之文稿　□撰寫中　□無
   專利：□已獲得　□申請中　■無
   技轉：□已技轉　□洽談中　■無
   其他：（以200字為限）

3. 請依學術成就、技術創新、社會影響等方面，評估研究成果之學術或應用價值（簡要敘述成果所代表之意義、價值、影響或進一步發展之可能性，以500字為限）
   過去二十年，台灣的肝硬化及肝癌死亡率節節攀升，目前肝硬化是台灣十大死因的第九名，而肝癌是台灣男性及女性主要癌症死亡原因的第二名。除了醫療外，藉由了解營養素在肝臟功能損傷、肝硬化或肝癌發生過程中所扮演的角色，透過給予營養素的介入輔助醫療，達到改善臨床結果是非常值得研究的方向。但是本研究卻發現給予肝損傷小鼠或肝硬化患者維生素B-6及GSH的補充無法降低氧化壓力，也無法增加其抗氧化能力。此研究結果應可提供動物及人體在肝損傷情況下，血漿維生素B-6及穀胱甘肽與氧化壓力及其相關的抗氧化能力關係的完整概念。

4. 主要發現
   本研究具有政策應用參考價值：■否　□是，建議提供機關
   （勾選「是」者，請列舉建議可提供施政參考之業務主管機關）
   本研究具影響公共利益之重大發現：□否　□是
   說明：（以150字為限）
   本研究未具影響公共利益之重大發現。