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The Effects of Concomitant use of Silymarin and Chemotherapy on Solid Tumors: A pilot randomized controlled trial

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ABSTRACT

Background: Anti-cancer potential of silymarin has been shown in cell culture. However, no pro¬spective clinical study has been conducted in this relation.

Methods: In a randomized double blind pilot study, we compared effects of addition of standard chemotherapy along with silymarin (420mg/day) versus placebo on clinical response of advanced tumors after three cycles of cisplatin-based chemotherapy.

Results: There was no significant differ¬ence in tumor size after three consecutive chemotherapy courses but a trend toward lower metastasis rate in chemotherapy + silymarin group. Concomitant use of sily¬marin and chemotherapy was very well tolerated but didn't significantly increase clinical response.

Conclusion: Due to the trend towards significantly lower metastasis in sily¬marin group, further study with larger sample size is needed to better clarify probable role of adjunctive therapy with silymarin in patients with solid tumors.

Keywords: silymarin-anticancer-solid tumor-chemotherapy

INTRODUCTION:

Silybum marianum, also known as milk thistle, a herbal plant from asteraceae families mainly comprises of flavonolignans including silybin, Isosilybin, silydianin, and silychristin[1, 2]. Silymarin is well known as a hepatoprotective agent in liver diseases such as alcoholic and non-alcoholic fatty liver, drug and toxin related liver disease[3]. Silymarin may also prevent liver function deterioration and hepatocellular carcinoma[3]. Preliminary animal and clinical studies have shown that silymarin flavonolignans or flavonoids may have protective effects concerning skin, lung, and breast cancers[4-6]. A series of well-done cell culture studies, mainly conducted by Agarwal et al, have shown antineoplastic properties for silymarin[7]. Pathways proposed of silymarin anti-neoplastic properties include apoptosis induction, suppression of inflammation, growth inhibition, anti-angiogenesis, cell cycle regulation, and metastasis inhibition[7-9].

Serum or plasma levels of vascular endothelial growth factor (VEGF) may might have prognostic value and anti-VEGF therapy might improve overall survival, progression free survival and overall response rate in gastrointestinal tumors[10]. Other studies also have shown that serum VEGF concentration may be useful in both diagnosis and prognosis of cancer in patients with ovarian carcinoma[11, 12]. However, routine measurement of VEGF is not standard of care.

Therefore, the aim of the present pilot study was to evaluate the effects of silymarin in comparison to placebo along with standard chemotherapy on clinical response and serum VEGF level in patients with solid tumors.

METHODS:

This study was a pilot randomized double-blinded placebo controlled trial conducted at Medical Oncology Department of Iran Cancer Institute from April 2013 until December 2014. The patients we randomized according to the computerized random number generator (1998-2017 RANDOM.ORG) .

Patients

The participants in the study, patients with upper gastrointestinal adenocarcinomas had to meet the following criteria: age>18 years old, Karnofsky performance status score more than70percent, receiving neo-adjuvant or palliative chemotherapy, have advanced measurable disease, undergoing cisplatin chemotherapy and signed informed consent. Patients with swallowing difficulties were excluded. Details of study exclusion criteria have been published before[13]. Because of very strict exclusion criteria and low recursions rate the authors randomized few patients with other solid tumors (mainly genitourinary tumors). They were taken 420 mg silymarin (Liver herb) or identical placebo tablets (Amin Pharmaceutical company, Isfahan, Iran) in 3 divided doses with meal, beginning 2 days before and ending 1 week after three consecutive chemotherapy cycles, including cisplatin and taxanes, and it had to be for at least six months after study inclusion. Flow chart of randomization has been shown in **figure 1**.

Ethics

The local Ethics Committee of Tehran University of Medical Sciences approved the study protocol. The proposal was approved by Iranian Registry of Clinical Trials committee with number of IRCT201207013043N6.

Measurements

Serum sample wasobtained at the beginning and the end of the study. Laboratory data, including white blood cells, hemoglobin, platelets count, liver and kidney function test and serum electrolytes, were measured daily during hospitalization. For VEGF (Bioassay technology laboratory, Shanghai china) samples were centrifuged at the rate of 10000 RPM

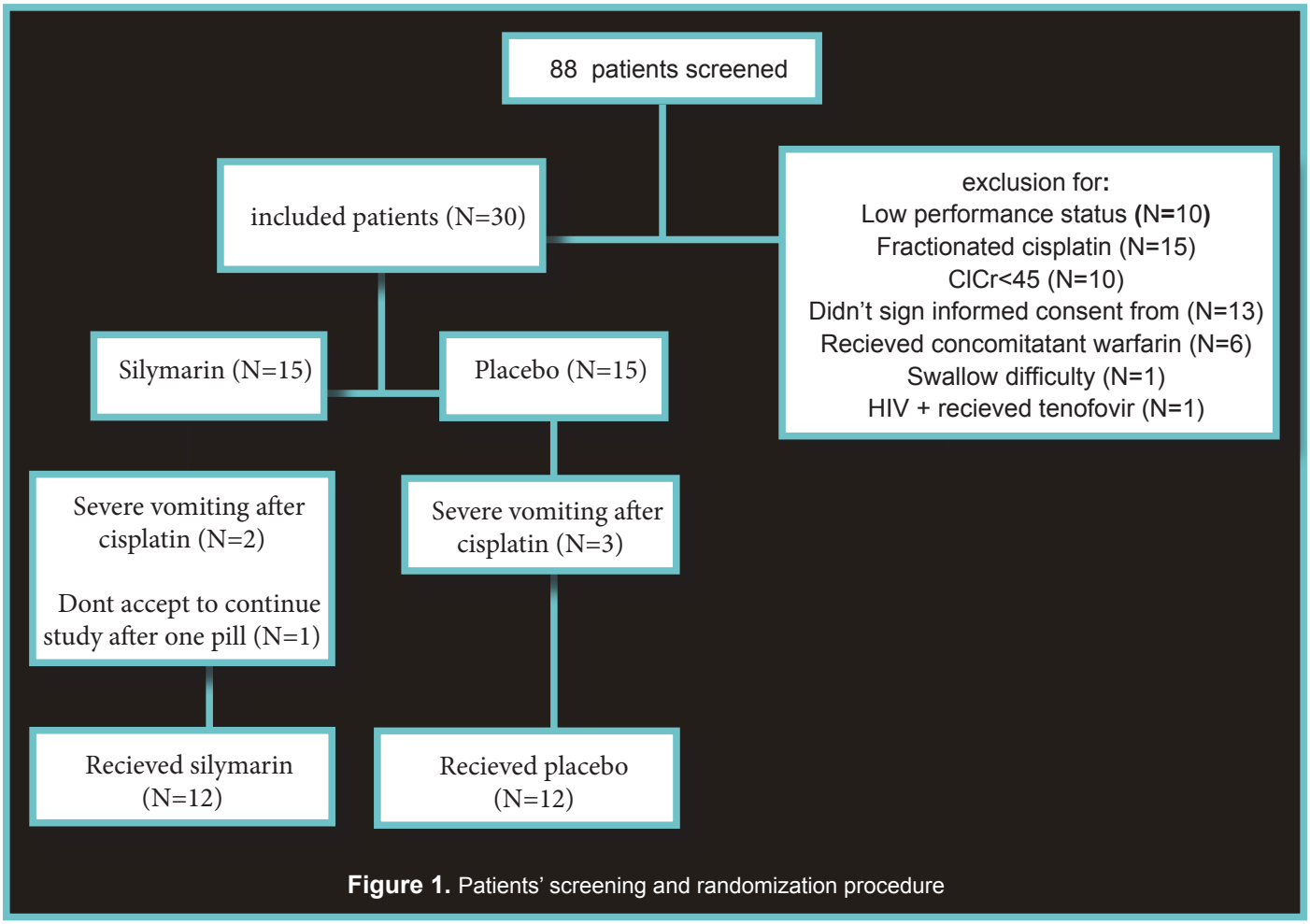

Figure 1. Patients' screening and randomization procedure

and were frozen at -80 C until reading. The range of detection assay and sensitivity were 20-6000ng/l and 10.25 ng/l respectively. Intra-assay Precision and Inter-assay Precision were 10% and 12% respectively.

Response to chemotherapy was checked by Chest, Abdominopelvic CT-Scan before the first cycle of chemotherapy and after the third cycle and evaluated based on RESIST criteria[14]. All responses (complete, partial and stable disease) were put together as clinical response.

Sample size

Due to lack of human data, the authors were not able to calculate the real sample size. Sample size of the present study was calculated for silymarin effects on cisplatin associated nephrotoxicity Published online[13].

Statistics

Shapiro-Wilk test was used to assess normality of data. The baseline characteristics and biochemical data of the patients were compared by two-unrelated samples t-tests. Fisher's exact test was used to compaire nominal variables. These analyses were done using SPSS software version 14 (SPSS Inc., Chicago, IL, USA). P values of less than 0.05 were considered as statistically significant. The relative risk

of progressive disease was checked by Cross-Tabulation in SPSS software.

RESULTS:

Twenty-four patients met the inclusion criteria (**Figure 1**). Upper gastrointestinal cancers (75%) were the most common ones in the study population, followed by ovary (21%) and one case of mesothelioma (**Table 1**). Baseline demographic characteristics of case and control groups were similar (**Table 2**).VEGF concentrations in the two groups have been shown in **table 3**. and the clinical response in **table 4**.

Although not statistically significant, the incidence of metastasis was lower in patients treated with silymarin (RR= 0.33 (CI 0.0834 to 1.3328, p=0.12). Other factors such as clinical response (p=0.6), relapse (p=0.9) and death (p=0.7) were similar concerning the two groups.

Patients' reported side effects during hospitalization and telephone follow-up were similar regarding silymarin and placebo groups. In view of liver function tests there were no difference regarding silymarin and placebo group.

Reported grade III and IV chemotherapy related side effects including anemia, leukopenia and dermatologic reaction, were the same in the two study groups (p=0.7). Toxicity and schedules of protocols are reported elsewhere[14].

Silymarin and placebo tablets were tolerated well without significant side effects.

DISCUSSION:

In the present study treatment with silymarin for three consecutive courses had no significant effect on serum VEGF concentration or clinical response

Table 1. Demographic characteristics of patients

Study group	Silymarin group	Placebo group	P
Age (years)	55.91 ± 8.6	51.16 ± 7.72	0.16
Female	25	50	0.40
Weight (Kg)	60.0 ± 8.8	57.0± 6.91	0.51
BSA(M^2)	60.0 ± 8.8	57.0± 6.91	0.51
Serum creatinine (mg/dl)	1.0 ± 0.2	0.9 ± 0.3	0.76
Albumin (g/dl)	4.100±0.492	4.042±0.380	0.74
White cell count (x10^3/mm^3)	7.92 ± 4.08	6.96 ± 4.97	0.24
Hemoglobin (g/dL)	11.0 ± 1.1	11.8 ± 1.0	0.89
Platelet count (x10^3/mm^3)	285.8 ± 139.5	246.3 ± 98.8	0.28
Serum AST (U/L)	24.3 ± 10.0	24.5 ± 6.0	0.96
Serum ALT (U/L)	22.3 ± 9.6	23.9 ± 8.8	0.66
Serum alkaline phosphatase (U/L)	482.0 ± 103.5	205.2 ± 46.1	0.75
Total bilirubin (mg/dl)	0.8 ±0.3	0.9± 0.1	0.21

Table 2. Chemotherapy regimens and malignancy		
Treatment regimen	Silymarin group	Placebo group
Cisplatin,docetaxel,5-FU (TCF)	5	7
Cisplatin,epirubicin,5-FU (ECF)	4	2
Cisplatin, pemetrexed	1	
Cisplatin, Gemcitabine	1	2
Cisplatin, ifosfamide, vinblastine		1
Cisplatin, paclitaxel	1	
Cancer type (number of patients) Upper GI Ovarian Mesothelioma	 9 2 1	 9 3 0

Table 3. VEGF concentration at initiation and end of study		
end of trial	Study initiation	Treatment group
212 (145-710)	282 (154-615)	Silymarin
320 (173-472)	457 (232-550)	Placebo
0.42	0.2	P

Table 4. Clinical response in silymarin and placebo group			
Treatment group	Silymarin (%)	Placebo (%)	P
Clinical Response	6 (50%)	4 (33%)	0.6
Metastasis	3 (25%)	7 (58%)	0.08
Palliative care	2 (16%)	1 (9%)	0.8
Death	1 (9%)	0	0.7

to chemotherapy. In human umbilical vein endothelial cells and colon cancer LoVo cell line, silymarin has shown a dose dependent effect on serum VEGF concentration[15, 16]. Silymarin inhibits angiogenesis in human umbilical vein endothelial cells at concentration of 50 µg/ml and higher within an hour [15]. Breast, ovarian and colon cancer cell lines are among in which silymarin may have anti-angiogenic effects[17]. Others have proposed that silymarin anti-angiogenicity is mediated mainly by VEGF-3

receptor down regulation and angiopoietin-2 up regulation[18]. Silybin may also inhibit epidermal growth factor receptor pathway[19]. It may also decrease cytoplasmic immune staining for VEGF[20]. However, because of low response and unresectability of tumors the authors were not able to assess tissue analysis after treatment.

Although not statistically significant, the rate of metastasis was lower in patients treated with silymarin. Anti-metastasis and anti-mutagenic properties of silymarin have been proposed for prostate, lung, breast, oral cancer and osteosarcoma[21-24]. More recently it has been proposed that silibinin can regulate adenosine 5'-monophosphate activated protein kinase (AMPK)/mammalian target of rapamycin (mTOR) and cyclin dependent kinase pathway in renal cell carcinoma and melanoma cells respectively[25, 26]. The results suggested that anti-proliferative effect of silymarin was mediated through cell cycle arrest at G1/S phase, cyclin-dependent kinase inactivation, apoptosis induction and inhibition of transcription of inflammatory factors[24, 26]. In addition, Kim et al reported that silibinin prevents tumor necrosis factor (TNF)-α induced matrix metalloproteinase-9 expression through the MEK/ERK pathway in gastric cancer cells in a dose dependent manner[27]. Furthermore, silymarin treated mice had lower chance of UV induced tumor, tumor volume and multiplicity28. Silymarin or its flavonolignans may act as a chemosensitizer in chemo-resistance prostate and gynecologic cancers and potentiate cisplatin antitumor properties[17]. Human and animal Pharmacokinetic studies have shown that silymarin concentrates in liver and is metabolized rapidly to its conjugated forms with elimination half-life of 30 minutes. In contrast, elimination half-life of silymarin in other tissues such as lung, stomach, skin and prostate is about 60-150 minutes[29]. Concomitant use of silymarin and chemotherapy in childhood acute lymphoblastic leukemia improves chemotherapy related hepatotoxicity without antagonizing chemotherapy. Silymarin treatment also potentiates vincristine antineoplastic properties[30]. Therefore, silymarin may be an effective chemo sensitizer or adjunctive treatment in patients with solid tumors. A phase one clinical trial also has shown that silymarin flavonolignan modified formulation, silybinphosphatidylcholine named as Siliphos®, is tolerated at dose of 2 gram/day in patients with hepatocellular carcinoma[31].

The results of the present study, although there was a decrease in metastasis rate, did not show positive effects of silymarin on assessed response parameters.

Lack of response may be due, in part, to the small sample size. Furthermore, the low serum and tissue concentration of silymarin may be another reason. Because there was no major toxicity of silimarin or no differences in toxicity in comparison to placebo, we recommend higher doses of silimarin be used in further studies.

In the present study there was some limitations the most important of which was the sample size calculated to assess silymarin effects on cisplatin-associated nephrotoxicity. Unfortunately, due to slow enrolment, the authors were unable to include patients with the similar tumor. This might have affected our data interpretations. However, response criteria were strict and the same. Due to strict inclusion and exclusion criteria, the researchers were unable to include more patients in the study (**figure 1**). A trend was found a trend toward significant lower metastasis in silymarin group. So, further study with a larger sample size is needed to better clarify the probable role of adjunctive therapy with silymarin in patients with solid tumors.

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