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The Prevalence and Short-Term Outcomes of Ventricular Dyssynchrony after Right Ventricular Pacing

Thipdhorn Aritajati[1], Kritsana Tipcome[2], Anusang Chitsomkasem[2], Nithi Tokavanich[2], Teetouch Ananwattanasuk[2], Padoemwut Teerawongsakul[2*]

[1]Division of Critical Care, Department of Medicine, Faculty of Medicine Vajira Hospital, Navamindradhiraj University, Bangkok, Thailand
[2]Division of Cardiology, Department of Medicine, Faculty of Medicine Vajira Hospital, Navamindradhiraj University, Bangkok, Thailand
Email: *padoemwut@nmu.ac.th

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Abstract

Objective: Long-term right ventricular pacing has been associated with an increased risk of heart failure and cardiomyopathy. The pathophysiology of cardiomyopathy associated with right ventricular pacing remains unclear. We aim to evaluate the burden and short-term outcomes of ventricular dyssynchrony after immediate permanent pacemaker implantation. Materials and Methods: This prospective cohort study examined consecutive patients who had permanent pacemaker implantation at Vajira Hospital in 2019. Left ventricular systolic function, specifically left ventricular ejection fraction (LVEF) and echocardiographic ventricular dyssynchrony parameters were assessed. The endpoints included the prevalence of ventricular dyssynchrony, new-onset cardiomyopathy, heart failure, and death. The correlation between QRS complex duration, the burden of ventricular pacing, and echocardiographic ventricular dyssynchrony was measured. Results: Thirty-six consecutive patients underwent pacemaker implantation. The prevalence of mechanical ventricular dyssynchrony was 22.2% using the interventricular conduction delay method, 41.7% using LV pre-ejection period method, and 11.1% using the septal posterior wall motion abnormality method. Electrical ventricular dyssynchrony was 86.1% and new-onset cardiomyopathy was 17.1% after 3 months of permanent pacemaker implantation. The right ventricular pacing of more than 20% was significantly associated with cardiomyopathy ($p < 0.022$) and heart failure (log-rank, $p = 0.049$) within 3 months. But heart failure was not associated with mechanical ventricular dyssynchrony parameters (log-rank, $p = 0.610$; hazard ratio [HR], 1.53; 95% confidence interval [CI], 0.29 - 7.96; p =

0.613 for IVMD and log-rank, p = 0.398; HR, 0.04; 95% CI, 0.01 - 3316.7 for SPWMD). Conclusion: Mechanical and electrical ventricular dyssynchrony are common findings in right ventricular pacing. High-burden right ventricular pacing after 3 months of permanent pacemaker implantation is often associated with cardiomyopathy and heart failure, but mechanical and electrical ventricular dyssynchrony does not predict a short-term decline in left ventricular systolic function and heart failure.

Keywords

Right Ventricular Pacing, Ventricular Dyssynchrony, Predictor, Correlation, Heart Failure, Cardiomyopathy, Left Ventricular Ejection Fraction Decline

1. Background

Symptomatic bradycardia and conduction block are common problems detected in elderly people. The standard treatment for symptomatic bradycardia is permanent pacemaker implantation (PPM) [1] [2]. Chronic right ventricular (RV) pacing has been associated with a deterioration in left ventricular (LV) systolic function [3]. Right ventricular pacing may produce mechanical and electrical ventricular dyssynchrony by activating the right ventricle to contract before the left ventricle (interventricular dyssynchrony) or the septum to contract before the lateral cardiac walls (intraventricular dyssynchrony). Asynchronous electrical activation of the ventricle causes left bundle branch block in traditional electrocardiography [4]. Septal and lateral wall out-phase contractions reduce stroke work and cause energy transfer from the contracting wall to the opposite relaxed wall. A deterioration in left ventricular function after chronic right ventricular pacing is known as pacing-induced cardiomyopathy (PICM). PICM has been recognized as a cause of heart failure in a patient with atrioventricular (AV) block [5]. A high rate of left ventricular pacing had been associated with left ventricular systolic dysfunction, frequently reported as PICM [6] [7]. Nonetheless, chronic RV pacing with preserved LV function may also be observed [8] [9].

Biventricular pacing or cardiac resynchronization therapy (CRT) is the coordination of right and left ventricular contractions. CRT is a proven treatment that improves congestive symptoms, quality of life and reduces mortality in patients with severe chronic heart failure with poor left ventricular systolic function [10]. Effective treatment for PICM is resynchronization of the right and left ventricle. A retrospective observational study of patients with PICM showed the reversal of cardiomyopathy with CRT [11].

Electrical ventricular dyssynchrony is indicated by a wide QRS complex duration, while mechanical ventricular dyssynchrony, lack of synchronization of both ventricles, can be detected by echocardiography. The prevalence of ventricular dyssynchrony in normal left ventricular systolic function and clinical cor-

relation in the short-term is not well established. Left ventricular systolic dysfunction and heart failure following permanent pacemaker implantation are controversial, especially in patient with good left ventricular systolic function. The primary objectives of this study are to evaluate the prevalence of mechanical and electrical dyssynchrony using simple echocardiography and electrocardiogram and to identify the short-term clinical effects following permanent pacemaker implantation in patients with preserved left ventricular systolic function.

2. Materials and Methods

2.1. Study Design

A prospective cohort study determines the prevalence of mechanical and electrical dyssynchrony using simple echocardiography parameters and QRS complex duration correlation.

All participants provided written informed consent. Study protocols were approved by the institutional review board from the Navamindradhiraj University and conducted in accordance with the ethical principles set out in the Declaration of Helsinki and the Good Clinical Practice Guidelines.

2.2. Study Population

We prospectively enrolled consecutive patients who had undergone single- or dual-chamber pacemaker implantation at a single tertiary care hospital located in Bangkok, Thailand, between February 2019 and November 2019. The inclusion criteria were as follows: 1) Of 18 years of age or older; 2) On permanent right ventricular pacing therapy; 3) Has a left ventricular ejection fraction (LVEF) of more than 35%. Patients who received biventricular pacing therapy were excluded from the trial.

2.3. Clinical Data and Measurement

Patients who met all the eligibility criteria were assessed by electrocardiography and echocardiography. The electrocardiography was performed before and after permanent pacemaker implantation to measure QRS complex duration and called QRS complex duration at pre-pacing and post-pacing periods; the left ventricular systolic function, specifically left ventricular ejection fraction (LVEF) was assessed at baseline and 3 months after device implantation. Mechanical dyssynchrony was measured and validated by the consensus of two cardiologists. Standard 12-lead electrocardiogram was recorded at 25 mm/s and 1 mV/cm with the PageWriter TC30 Cardiograph (Philips, the Netherlands). Intrinsic and paced QRS complex duration measurements were performed in digital format from the beginning of the Q wave to the end of the S wave.

Collected clinical data was assessed, including patient demographic characteristics, previous disease before permanent pacemaker implantation, electrocardiographic and echocardiographic findings before permanent pacemaker im-

plantation, indications for permanent pacemaker implantation, procedural outcomes after permanent pacemaker implantation, post-permanent pacemaker implantation follow-up device diagnostics, and electrocardiographic and echocardiographic findings during right ventricular pacing after permanent pacemaker implantation.

Heart failure within 3 months were collected for survival analysis. Heart failure was categorized into outpatient care (up-titrating diuretic due to clinical heart failure or starting a new loop diuretic due to clinical heart failure) or hospitalization.

3. Definition

3.1. Mechanical Dyssynchrony

Mechanical dyssynchrony can be evaluated by echocardiography as outlined below.

Interventricular dyssynchrony was measured by a discordance between the time of RV and LV contraction. On the other hand, pulse-wave doppler images of aortic and pulmonary flow velocities were utilized to measure interventricular mechanical delay (IVMD). IVMD included a recording of the LV outflow tract from an apical five-chamber view and the RV outflow tract from a parasternal short-axis view of the pulmonary artery. The time differences were utilized between electrocardiogram-derived Q wave onset and the onset of LV outflow and the time between the Q wave onset and the onset of RV outflow [12] [13].

Intraventricular mechanical dyssynchrony is considered dyssynchrony within the LV. M-mode septal to posterior wall motion abnormality (SPWMD) was calculated as the difference in the timing of both septal and posterior wall contractions. The M-mode cursor was positioned perpendicular to the septum and posterior wall at the base of the LV in a parasternal axis view. SPWMD was the calculated difference between the time from onset of the electrocardiogram-derived Q wave to the peak posterior displacement of the septum; it is also known as the time from the onset of QRS to the peak systolic displacement of the posterior wall [13] [14] [15].

3.2. Electrical Dyssynchrony

Electrical dyssynchrony was defined as a QRS complex duration of >130 msec.

3.3. Pacemaker-Induced Cardiomyopathy

Pacemaker-induced cardiomyopathy after permanent pacemaker implantation was defined as a decline LVEF from baseline more than 10%.

3.4. Trial Endpoints

The primary endpoint was the prevalence of electrical and mechanical dyssynchrony. The secondary outcomes were the association of ventricular dyssynchrony and adverse clinical outcomes (heart failure and new-onset LV systolic

dysfunction) at 3 months, the correlation between the burden of RV pacing and adverse clinical outcomes (heart failure and new-onset left ventricular systolic dysfunction), and the correlation between electrical and mechanical dyssynchrony.

3.5. Statistical Analysis

Continuous variables were reported as mean and standard deviation for normally distributed variables and median and interquartile range (IQR) for variables with a non-normal distribution. Categorical data were presented as frequencies and percentages. Independent t-tests, Pearson's correlation coefficient, and the Mann-Whitney U test were used as appropriate. A p-value of <0.05 was considered statistically significant. Survival analysis time-to-event outcomes were presented as cumulative events (Kaplan-Meier estimate for endpoints including new-onset LV dysfunction and heart failure). Data was analyzed using SPSS software version 22 for Windows (SPSS Inc., Chicago).

4. Results

Forty-four patients were enrolled in this study, and five were lost to follow-up. Thirty-nine patients were admitted for permanent pacemaker implantation. The three patients were excluded due to an incomplete 3-month follow-up. Total patients with completed the follow-up were 36 patients (81.1%). The baseline characteristics of patients were listed in Table 1. More than half of the 36 participants included in the analysis were male (51.2%) and mean age 69.89 ± 15.72 years. A total of 33.3% of patients had diabetes mellitus, 72.2% with hypertension, 69.4% with dyslipidemia, 44.4% with atrial arrhythmia, 27.8% with ischemic heart disease, and 30.6% with chronic kidney disease. Eighteen patients (43.9%) were found to have sinus node dysfunction and 16 patients (39%) with AV nodal disease.

The prevalence of mechanical dyssynchrony estimated by IVMD, LV preejection period (LVPEP), and SPWMD was at 22.2%, 41.7%, and 11.1%, respectively. Electrical dyssynchrony estimated by a QRS complex duration of more than 130 msec occurred in 86.1% of patients.

Six patients (16.7%) developed PICM. Patients with PICM had a significant reduction in LVEF compared with non-PICM group (16.4% ± 5.8% vs. 0.5% ± 0.9%, respectively; p = 0.001; Table 2). LVEF before implantation was not significantly different between patients in the PICM and non-PICM groups (66.1% ± 9.7% vs. 66.2% ± 13.4%; p = 0.993). The PICM group had a higher percentage for RV pacing (98.5%, IQR = 89.0% - 100.0% vs. 11.3%, IQR = 1.6% - 97.0%; p = 0.022). High-burden RV pacing (>20%) was found to be significantly correlated with PICM. LVEF in patients in the PICM group significantly changed at 1 month (p = 0.024) and 3 months (p = 0.049) after implantation. However, the indications for permanent pacemaker implantation were not associated with PICM (sinus node dysfunction, p = 0.67; AV nodal dysfunction, p = 0.18). The

Table 1. Baseline characteristics of study participants.

Variables	Total (n = 36)	Non-PICM (n = 30)	PICM (n = 6)	p-value
Postprocedural CHB	14 (38.9%)	10 (33.3%)	4 (66.7%)	0.181
Age (years)	71.4 ± 15.4	71.1 ± 15.7	72.5 ± 15.0	0.851
Sex: Male	20 (55.6%)	16 (53.3%)	4 (66.7%)	0.672
Diabetes mellitus	12 (33.3%)	11 (36.7%)	1 (16.7%)	0.640
Hypertension	26 (72.2%)	22 (73.3%)	4 (66.7%)	1
Dyslipidemia	25 (69.4%)	21 (70.0%)	4 (66.7%)	1
Atrial arrhythmia	16 (44.4%)	13 (43.3%)	3 (50.0%)	1
Ischemic heart disease	10 (27.8%)	7 (23.3%)	3 (50.0%)	0.317
Chronic kidney disease	11 (30.6%)	10 (33.3%)	1 (16.7%)	0.643
Intrinsic QRS duration (msec)	107.9 ± 24.8	106.4 ± 25.9	121.7 ± 21.1	0.187
Paced QRS duration (msec)	151.6 ± 35.0	154.4 ± 27.0	150.7 ± 19.1	0.750
Pacemaker mode				
DDD	25 (69.4%)	21 (70.0%)	6 (66.7%)	1
VVI	11 (30.6%)	9 (30.0%)	2 (33.3%)	1
Rate adaptative	12 (33.3%)	10 (33.3%)	2 (33.3%)	1
Atrial pacing rate (%)	30.6 (6.0 - 49.5)	32.9 (6.0 - 53.0)	18.0 (5.0 - 31.5)	0.603
RV paced (%)	50.2 (2.0 - 98.0)	11.3 (1.6 - 97.0)	98.5 (89.0 - 100.0)	0.022

Values are presented as mean ± standard deviation (SD) or median (IQR) and n (%). The p-value corresponds to the independent t-test or Mann-Whitney U test and Fisher's exact test.

Table 2. Post-ventricular pacing characteristics.

Variables	Total (n = 36)	Non-PICM (n = 30)	PICM (n = 6)	p-value
Electrical dyssynchrony				
QRS duration ≥130 ms	31 (86.1%)	26 (86.7%)	5 (83.3%)	0.829
Mechanical dyssynchrony				
IVMD ≥40 ms	8 (22.9%)	6 (20.7%)	2 (33.3%)	0.516
LVPEP ≥140 ms	15 (42.9%)	11 (37.9%)	4 (66.7%)	0.207
SPWMD ≥130 ms	4 (11.8%)	2 (7.1%)	2 (33.3%)	0.071
RV paced (%)				
≥20% RV paced	20 (55.6%)	14 (46.7%)	6 (100.0%)	0.024
≥40% RV paced	18 (50.0%)	12 (40.0%)	6 (100.0%)	0.019
Pre-procedural LVEF	65.2 ± 13.0	66.2 ± 13.4	66.1 ± 9.7	0.993
Post-procedural LVEF at 3 months	63.0 ± 15.0	65.6 ± 14.7	49.7 ± 8.1	0.015
Post-procedural LVEF <55%	9 (25.0%)	5 (16.7%)	4 (66.7%)	0.025
Difference between pre and post LVEF	3.1 ± 8.4	0.5 ± 5.9	16.4 ± 5.8	<0.001
Heart failure	10 (27.8%)	7 (23.3%)	3 (50.0%)	0.317

Values are presented as mean ± standard deviation (SD) or median (IQR) and n (%). The p-value corresponds to the independent t-test or the Mann-Whitney U test and Fisher's exact test.

pacemaker mode was not associated with PICM (Table 1).

All ventricular dyssynchrony parameters from echocardiography and electrocardiography were not found to be associated with PICM (Table 2). The electrical dyssynchrony, specifically wide QRS complex duration of more than 130 msec was 83.3% in the PICM group and 86.7% in the non-PICM group. The electrical dyssynchrony rate tended to be higher in PICM but there was no statistical significance associated with PICM ($p = 0.829$). In approximately one-third of patients with PICM showed interventricular mechanical delay (IVMD) more than 40 msec (PICM group, 33.3% vs. non-PICM group, 20.7%; $p = 0.516$). While a Left Ventricular Pre-Ejection Period (LVPEP) more than 140 msec was almost twice as common in patient with PICM than non-PICM (PICM group, 66.7% vs. non-PICM group, 37.9%; $p = 0.207$). Similarly, septal posterior wall dyssynchrony (SPWMD) >130 msec was four times more common in PICM group than in the non-PICM group. (PICM group, 33.3% vs. non-PICM group, 7.1%; $p = 0.071$).

During RV pacing, ventricular dyssynchrony was observed. Electrical dyssynchrony was significantly changed after RV pacing (intrinsic QRS duration = 102.0 msec, IQR = 91.0 - 124.0 msec vs. paced QRS duration = 157.5 msec, IQR = 140.5 - 171.5 msec; $p < 0.001$). Interventricular conduction delay changed significantly after RV pacing (mean pre-pacing IVMD, 8.0 ± 23.6 vs. mean post-pacing IVMD, 23.7 ± 26.1; $p < 0.01$). LVPEP and SPWMD were not significantly different after RV pacing. The QRS complex during RV pacing correlated with IVMD and LVPEP ($r = 0.361$, $p = 0.024$ and $r = 0.315$, $p = 0.028$; Figure 1).

A Kaplan-Meier survival curve of cumulative heart failure demonstrated a clinically association between heart failure and a high-burden of right ventricular pacing more than 20% within 3 months (log-rank, $p = 0.086$; Figure 2). Mechanical dyssynchrony was estimated using IVMD, LVPEP, and SPWMD and was not significantly associated with heart failure (log-rank, $p = 0.610$ for IVMD; $p = 0.112$ for LVPEP; $p = 0.398$ for SPWMD). Pacing-induced cardiomyopathy was associated with clinical heart failure but was not significantly different between patients in the PICM and non-PICM groups (23.3% vs. 50%; $p = 0.317$).

5. Discussion

Ventricular dyssynchrony was common in the RV pacing and may contribute to the worsening of LV systolic function. Because of the small number of study participants and the short follow-up period, mechanical and electrical dyssynchrony parameters could not predict short-term heart failure, cardiomyopathy and the correlation between ventricular dyssynchrony and ventricular systolic dysfunction.

From our study, the patients with a LVEF of more than 35% and high-burden RV pacing demonstrated new-onset cardiomyopathy and heart failure within a 3-month follow-up after permanent pacemaker implantation. Patients with a LVEF of 35% or lower were excluded due to strong recommendation for biventricular pacing. While LVEF is between 35% to 50%, right ventricular pacing or

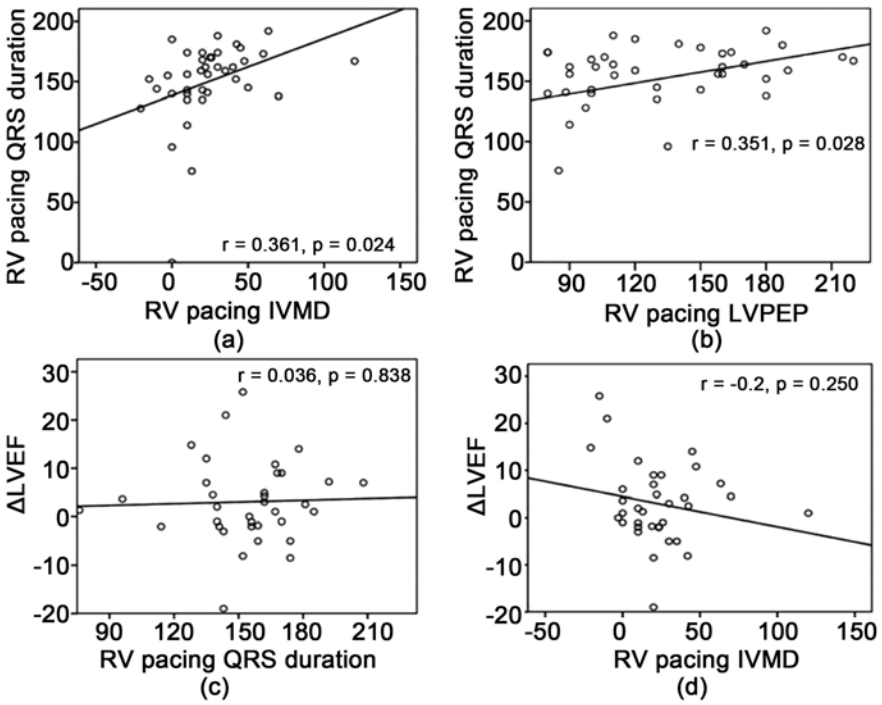

Figure 1. The correlation between mechanical, electrical ventricular dyssynchrony and difference in left ventricular systolic function at 3 months after permanent pacemaker implantation. (a) The correlation between RV pacing QRS complex duration and interventricular mechanical delay (IVMD). (b) The correlation between RV pacing QRS complex duration and Left Ventricular Pre-Ejection Period (LVPEP). (c) The correlation between RV pacing QRS complex duration and pre and post PPM difference in left ventricular ejection fraction (LVEF) implantation. (d) The correlation between pre and post PPM implantation difference in left ventricular ejection fraction (LVEF) and interventricular mechanical delay (IVMD).

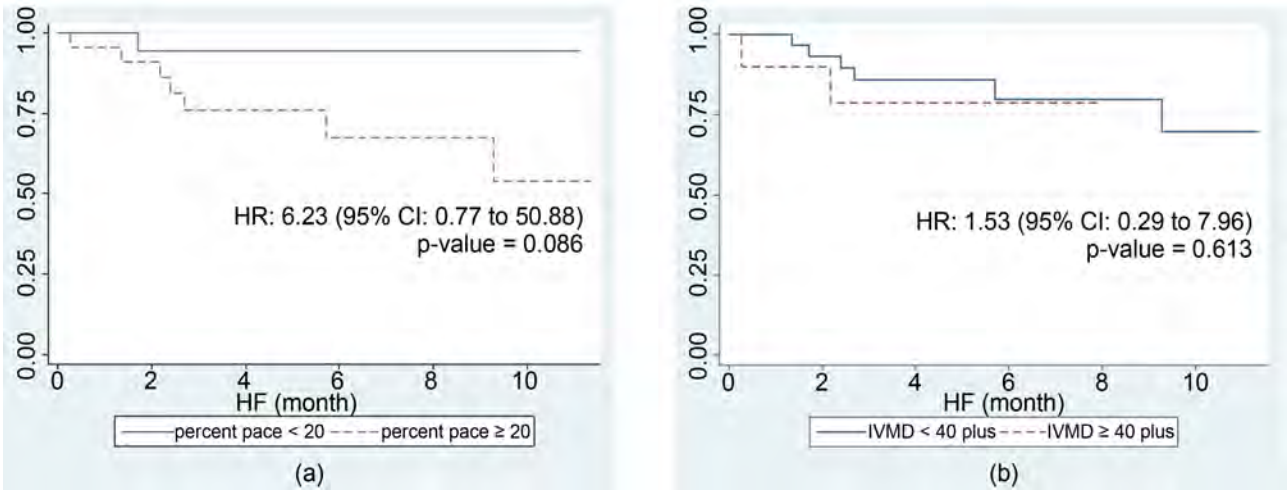

Figure 2. Cumulative incidence of heart failure. Kaplan-Meier curves show the cumulative incidence of heart failure with (a) Burden of RV pacing. (b) Mechanical ventricular dyssynchrony, particularly interventricular mechanical delay (IVMD).

biventricular pacing were therapeutic options. This data reveals the prevalence and time course of PICM and heart failure in the presence of high-burden RV

pacing, suggesting that the adverse clinical effects of PICM and heart failure may occur quickly.

Several studies have reported that RV pacing is significantly related to PICM and heart failure. For example, the Pacing to Avoid Cardiac Enlargement trial randomized 86 patients to an RV pacing group. In this group, mean LVEF decreased from 61.5% to 54.8% ($p < 0.01$) at 12 months. In contrast, in the biventricular pacing group, LVEF and ventricular volumes remained stable when compared with baseline [16]. Acute deterioration of LV systolic function was detected in the other study. Twelve participants with a dual chamber pacemaker implantation, normal LV function and physiological AV nodal conduction were examined using the serial gait blood pool technique. LVEF decreased from 66.5% ± 4.5% to 52.9% ± 8.3%; ($p < 0.0001$) after 1 week of RV pacing. After cessation of pacing at 32 hours, LVEF increased to 62.9% ± 7.6% ($p = 0.11$) compared with baseline [17]. These findings were similar to our results showing the occurrence of PICM within a short follow-up period. The threshold of RV pacing also observed in our study, in an analysis of patients receiving a permanent pacemaker from 2000 to 2014 for complete heart block with a LVEF of >50%, 823 patients (12.3%) developed PICM over a mean follow-up period of 4.3 ± 3.9 years. The PICM group was significantly associated with the RV pacing of more than 20% [18]. From Merchant Faisal M and et al., acute heart failure after RV pacing was reported in the group with complete AV block within 6 months (HR = 1.62, 95% CI 1.48 - 1.79; $p < 0.001$) [19]. Accordingly, these studies supported our results. RV pacing might be contributing to acute adverse hemodynamic events, which in turn result in PICM and acute heart failure. Multiple clinical trials have demonstrated reverse remodeling after resynchronized right and left ventricular stimulation. CRT showed improved morbidity, congestive heart failure, and mortality [20]. Thus, physiologic pacing, such as His-bundle pacing or biventricular pacing, has been recommended for high-burden RV pacing to avoid PICM and heart failure [2].

RV pacing did not affect some patients in this study. On the other hand, a previous cohort study reported infrequent development of left ventricular systolic dysfunction in pacemaker recipients with predominantly normal LVEF [21]. Many factors cause PICM and heart failure, one of which is high-burden RV pacing. After multivariable data analysis, we did not detect a significant correlation between cardiomyopathy and other factors. Considering that the sample size was small and follow-up duration short, the multivariate analysis was limited. In this study, the PICM group had a higher rate of ischemic heart disease compared with the non-PICM group (50% vs. 23.3%) but not statistically significant. Ischemic heart disease accelerates or precipitates LV systolic dysfunction and heart failure. Atrial fibrillation, age, gender, and pre-existing valvular dysfunction were not associated with early onset heart failure and PICM in our study, although the real impact of RV pacing is difficult to detect due to many confounding factors. Additional studies are needed to identify individuals most

susceptible to the adverse effects of RV pacing and to determine who might benefit from biventricular pacing.

6. Study Limitations

Our study has several important limitations that should be noted. This study had a small sample size that could limit the power of multivariate analysis. Due to the short follow-up duration, the low rates of adverse events, such as left ventricular systolic dysfunction and heart failure, made it impossible to differentiate ventricular dyssynchrony. We suggested further study should be longer follow up. Due to small sample size and short duration follow-up time, the heart failure patients in this study were defined with first onset heart failure. The quality of life such as functional class, six-minute walk test, and pulmonary hypertension parameters should be collected as evidence of heart failure. Our study did not record other echocardiographic information about left ventricular remodeling, such as diastolic and systolic left ventricular internal diameter or global longitudinal strain pattern, which could represent the severity of cardiac remodeling. Echocardiographic strain patterns were not included in our study due to facility limitations, which might have been helpful in detecting LV dyssynchrony early and with good specificity.

7. Clinical Implications

Ventricular dyssynchrony is common after permanent pacemaker implantation. Early ventricular systolic dysfunction can occur in a short duration. If a patient is clinically suspected of having LV systolic dysfunction, such as dyspnea or clinical heart failure, LV systolic function assessment should be carried out. In high-risk groups, an evaluation of LV systolic function may be beneficial for PICM. Upgrading to CRT can reverse ventricular systolic dysfunction.

8. Conclusion

Mechanical and electrical dyssynchrony is a common finding after RV pacing. New-onset cardiomyopathy is significantly associated with high-burden RV pacing (>20%) within 3 months after implantation. High-burden RV pacing is also found to be contributing to heart failure. Future studies should look into identifying individuals most susceptible to the adverse effects of RV pacing, a way to determine which patients might benefit from biventricular pacing or his bundle pacing.

Acknowledgements

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Potential Conflicts of Interest

The authors declare no conflict of interest.

References

[1] Kusumoto, F.M., Schoenfeld, M.H., Barrett, C., *et al.* (2018) ACC/AHA/HRS Guideline on the Evaluation and Management of Patients with Bradycardia and Cardiac Conduction Delay. *Heart Rhythm*, **16**, E128-E226. https://doi.org/10.1016/j.hrthm.2018.10.037

[2] Kusumoto, F.M., Schoenfeld, M.H., Barrett, C., Edgerton, J.R., Ellenbogen, K.A., Gold, M.R., *et al.* (2019) ACC/AHA/HRS Guideline on the Evaluation and Management of Patients With Bradycardia and Cardiac Conduction Delay: A Report of the American College of Cardiology/American Heart Association Task Force on Clinical Practice Guidelines and the Heart Rhythm Society. *Circulation*, **140**, e382-e482.

[3] Wiggers, C.J. (1925) The Muscular Reactions of The Mammalian Ventricles to Artificial Surface Stimuli. *American Journal of Physiology-Legacy Content*, **73**, 346-378. https://doi.org/10.1152/ajplegacy.1925.73.2.346

[4] Grines, C.L., Bashore, T.M., Boudoulas, H., Olson, S., Shafer, P. and Wooley, C.F. (1989) Functional Abnormalities in Isolated Left Bundle Branch Block. The Effect of Interventricular Asynchrony. *Circulation*, **79**, 845-853. https://doi.org/10.1161/01.CIR.79.4.845

[5] Sweeney, M.O. and Hellkamp, A.S. (2006) Heart Failure during Cardiac Pacing. *Circulation*, **113**, 2082-2088. https://doi.org/10.1161/CIRCULATIONAHA.105.608356

[6] Jessup, L.M., Chandler, S.L. and Kay, H.R. (1987) Clinical Determinants of Mortality in Chronic Congestive Heart Failure Secondary to Idiopathic Dilated or to Ischemic Cardiomyopathy. *American Journal of Cardiology*, **59**, 634-638. https://doi.org/10.1016/0002-9149(87)91183-0

[7] The DAVID Trial Investigators (2002) Dual-Chamber Pacing or Ventricular Backup Pacing in Patients with an Implantable Defibrillator: The Dual Chamber and VVI Implantable Defibrillator (DAVID) Trial. *JAMA*, **288**, 3115-3123. https://doi.org/10.1001/jama.288.24.3115

[8] Zhang, X.H., Chen, H., Siu, C.W., Yiu, K.H., Chan, W.S., Lee, K.L., *et al.* (2008) New-Onset Heart Failure after Permanent Right Ventricular Apical Pacing in Patients with Acquired High-Grade Atrioventricular Block and Normal Left Ventricular Function. *Journal of Cardiovascular Electrophysiology*, **19**, 136-141. https://doi.org/10.1111/j.1540-8167.2007.01014.x

[9] Sweeney, M.O., Hellkamp, A.S., Ellenbogen, K.A., Greenspon, A.J., Freedman, R.A., Lee, K.L., *et al.* (2003) Adverse Effect of Ventricular Pacing on Heart Failure and Atrial Fibrillation Among Patients With Normal Baseline QRS Duration in a Clinical Trial of Pacemaker Therapy for Sinus Node Dysfunction. *Circulation*, **107**, 2932-2937. https://doi.org/10.1161/01.CIR.0000072769.17295.B1

[10] Cleland, J.G.F., Daubert, J.C., Erdmann, E., Freemantle, N., Gras, D., Kappenberger, L., *et al.* (2005) The Effect of Cardiac Resynchronization on Morbidity and Mortality in Heart Failure. *New England Journal of Medicine*, **352**, 1539-1549. https://doi.org/10.1056/NEJMoa050496

[11] Khurshid, S., Obeng-Gyimah, E., Supple, G.E., Schaller, R., Lin, D., Owens, A.T., *et al.* (2018) Reversal of Pacing-Induced Cardiomyopathy Following Cardiac Resynchronization Therapy. *JACC: Clinical Electrophysiology*, **4**, 168-177. https://doi.org/10.1016/j.jacep.2017.10.002

[12] Waggoner, A.D. and Adams, D.B. (2007) Cardiac Resynchronization Therapy and the Emerging Role of Echocardiography (Part 1): Indications and Results from

Current Studies. *Journal of the American Society of Echocardiography*, **20**, 70-75. https://doi.org/10.1016/j.echo.2005.10.021

[13] Agler, D.A., Adams, D.B. and Wanggoner, A.D. (2007) Cardiac Resynchronization Therapy and the Emerging Role of Echocardiography (Part 2): The Comprehensive Examination. *Journal of the American Society of Echocardiography*, **20**, 76-90. https://doi.org/10.1016/j.echo.2005.11.009

[14] Pitzalis, M.V., Iacoviello, M., Romito, R., Massari, F., Rizzon, B., Luzzi, G., *et al.* (2002) Cardiac Resynchronization Therapy Tailored by Echocardiographic Evaluation of Ventricular Asynchrony. *Journal of the American College of Cardiology*, **40**, 1615-1622. https://doi.org/10.1016/S0735-1097(02)02337-9

[15] Pitzalis, M.V., Iacoviello, M., Romito, R., Guida, P., De Tommasi, E., Luzzi, G., *et al.* (2005) Ventricular Asynchrony Predicts a Better Outcome in Patients with Chronic Heart Failure Receiving Cardiac Resynchronization Therapy. *Journal of the American College of Cardiology*, **45**, 65-69. https://doi.org/10.1016/j.jacc.2004.09.058

[16] Fung, J.W., Chan, J.Y., Omar, R., Hussin, A., Zhang, Q., Yip, G., *et al.* (2007) The Pacing to Avoid Cardiac Enlargement (PACE) Trial: Clinical Background, Rationale, Design, and Implementation. *Journal of Cardiovascular Electrophysiology*, **18**, 735-739. https://doi.org/10.1111/j.1540-8167.2007.00862.x

[17] Nahlawi, M., Waligora, M., Spies, S.M., Bonow, R.O., Kadish, A.H. and Goldberger, J.J. (2004) Left Ventricular Function during and after Right Ventricular Pacing. *Journal of the American College of Cardiology*, **44**, 1883-1888. https://doi.org/10.1016/j.jacc.2004.06.074

[18] Erich, L., Kiehl, T.M.M., Kumar, R., Gumber, D., Kwon, D.H., Rickard, J.W., Kanj, M., Wazni, O.M., Saliba, W.I., Varma, N., Wilkoff, B.L. and Cantillon, D.J. (2016) Incidence and Predictors of Right Ventricular Pacing-Induced Cardiomyopathy in Patients with Complete Atrioventricular Block and Preserved Left Ventricular Systolic Function. *Heart Rhythm*, **13**, 2272-2278. https://doi.org/10.1016/j.hrthm.2016.09.027

[19] Merchant, F.M., Hoskins, M.H., Musat, D.L., Prillinger, J.B., Roberts, G.J., Nabutovsky, Y., *et al.* (2017) Incidence and Time Course for Developing Heart Failure With High-Burden Right Ventricular Pacing. *Circulation*: *Cardiovascular Quality and Outcomes*, **10**, e003564. https://doi.org/10.1161/CIRCOUTCOMES.117.003564

[20] Rivero-Ayerza, M., Theuns, D.A.M.J., Garcia-Garcia, H.M., Boersma, E., Simoons, M. and Jordaens, L.J. (2006) Effects of Cardiac Resynchronization Therapy on Overall Mortality and Mode of Death: A Meta-Analysis of Randomized Controlled Trials. *European Heart Journal*, **27**, 2682-2688. https://doi.org/10.1093/eurheartj/ehl203

[21] Ebert, M., Jander, N., Minners, J., Blum, T., Doering, M., Bollmann, A., *et al.* (2016) Long-Term Impact of Right Ventricular Pacing on Left Ventricular Systolic Function in Pacemaker Recipients With Preserved Ejection Fraction: Results From a Large Single-Center Registry. *Journal of the American Heart Association*, **5**, e003485. https://doi.org/10.1161/JAHA.116.003485

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Accuracy of Noncontrast CT Brain in Detection of Cerebral Venous Sinus Thrombosis

Pipat Chiewvit[1*], Siri-on Tritrakarn[1], Yongchai Nilanont[2]

[1]Department of Radiology, Faculty of Medicine Siriraj Hospital, Mahidol University, Bangkok, Thailand
[2]Division of Neurology, Department of Medicine, Faculty of Medicine Siriraj Hospital, Mahidol University, Bangkok, Thailand
Email: *pipat8999@gmail.com

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Abstract

Background and Purpose: Increasing concern of cerebral venous thrombosis due to treatable and curable causes of stroke. The diagnosis of cerebral venous sinus thrombosis is challenged due to nonspecific clinical symptomatology. Patients may present at an emergency room with a variety of neurological conditions such as severe headache, weakness, seizure, etc. Neuroimaging, particularly noncontrast cranial computed tomography (NCCT), is an investigation of choice in differentiation and triage the patients for further treatment. CT is sensitive in the detection of acute thrombosis or blood clots in all regions of the body. We hypothesize that NCCT might be sensitive to diagnose cerebral venous thrombosis immediately. **Materials and Methods:** Retrospectively review the electronic database of our patients, there are 27 patients with cerebral sinus venous thrombosis (SVT) and 4 patients with cerebral deep venous thrombosis (DVT). Other 79 patients present with clinically diagnosed cerebral venous thrombosis but the final result can exclude cerebral venous thrombosis. We use MR imaging and CT venography as the gold standard. Independently reviewed by two neuroradiologists for CT direct sign and CT indirect signs that suggest SVT or DVT. CT direct signs for SVT and DVT are the presence of hyperdensity in the sinus venous or deep venous system (cord sign, attenuated vein sign) and CT indirect signs are the changes in brain parenchyma (brain edema, hemorrhagic infarction). **Results:** Sensitivity and specificity of NCCT in detection attenuated vein sign and diagnosis DVT are 75% and 100% whereas the sensitivity and specificity of NCCT in detection cord sign and diagnosis SVT are 43.8% and 99.7% as respectively. **Conclusions:** NCCT might not sensitive in detection of SVT without CT direct sign which needs further investigation. However, NCCT might beneficial for emergency conditions such as DVT patients, cortical vein thrombosis and also in SVT patients with the positive CT direct sign.

Keywords

Noncontrast CT, CT Brain, Cerebral Venous Sinus Thrombosis Cord Sign, Hyperattenuate Sign, CVT

1. Introduction

Cerebral venous thrombosis (CVT) is one of the causes of stroke even though less common comparable to stroke from arterial thrombosis. It still represents the common cause of stroke in young adults. Nowadays, CVT is increasingly encountered in daily practice.

Early diagnosis of cerebral venous thrombosis is important for the early treatment which can improve morbidity and mortality of the patients. Clinical symptoms of CVT are nonspecific [1] [2] with a wide range of severity such as headache, nausea, vomiting, dizziness up to neurological deficits and epilepsy [3] [4].

In contrast to the patients with stroke from arterial thrombosis, the symptoms of CVT patients are not present in the acute stage but slow progression [5] [6] [7]. Severe headache is the most common symptom which is counts as 74% - 90% of CVT patients and nonspecific [3] [8]. Early diagnosis of CVT should be in the lists of differential diagnoses whenever encountered in the young patients with unusual severe headache, with clinical arterial stroke and absence of risk factors, with intracranial hypertension or patients with hemorrhagic infarction on NCCT. The most common locations of intracranial dural venous sinuses are superior sagittal sinus followed by transverse sinus and sigmoid sinus as respectively [3] [4] [9].

Although CVT is not common, altogether with this condition awareness and new model of imaging CT scanner with rapid scan time, thin slices section, high resolution, the incidence of CVT is increase. Shorter time from onset of symptoms to diagnosis is with average of 7 days [3] [8].

Magnetic Resonance Imaging (MRI) and Magnetic Resonance venography (MRV) are the most sensitive imaging technique in diagnosis CVT [2] [8] [10]. The disadvantages of MRI are such as time consuming imaging technique, need patient cooperation particularly in the emergency condition [2] [6] [11]. So, multidetector computed tomography with venography (MDCTV) is of increased utility as the imaging modality in diagnosis CVT after inconclusive by NCCT.

NCCT can be performed faster and is suitable in the neuroemegency condition, still use as the first line of imaging modality in most centers. The radiologic patterns of NCCT in diagnosis of CVT (both SVT and DVT) are divided into direct signs and indirect signs. "Direct sign" refers to the visualization of the acute intraluminal blood clot or acute thrombosis that developed in the cranial venous system. If the thrombus formed within a week (acute stage), the attenuation value or CT density is higher than brain density and locates along course

venous sinus, cortical veins or deep vein [5] [6]. After one to two weeks (subacute stage), the thrombus is getting decreased density to the same level of brain (isodensity) or lower than the brain (hypodensity) which might not be easily diagnosed by NCCT. "Indirect sign" refers to the brain parenchyma density changes resulting from cerebral venous thrombosis which could be brain edema or hemorrhagic infarction, etc. [6].

Previous study reports that the sensitivity and specificity of NCCT in detection direct sign in cerebral venous thrombosis for SVT is poor [7] which is contrary to our experiences in daily practice. The purpose of this study is to reevaluation the usefulness of NCCT in diagnosis CVT.

2. Material and Method

This study was approved by institutional review board committee and performed in Siriraj hospital.

2.1. Definitions

We defined the patients with cerebral venous thrombosis into two subgroups such as sinus venous thrombosis (SVT) which represents thrombosis in course dural sinus such as superior sagittal sinus, inferior sagittal sinus, transverse sinus, sigmoid sinus and deep venous thrombosis (DVT) which thrombosis is detected in those following veins as thalamostriate vein (TSV), internal cerebral veins (ICV), basal vein of Rosenthal (BVR), great vein of Galen (VG).

2.2. Subjects

Retrospectively review of the medical records of the patients who was diagnosed as cerebral venous thrombosis with undergone NCCT, CT venography (CTV) or MRV in past 3 years between 2009-2012.

Inclusion criteria: 1) NCCT must be performed; 2) Patients must have one of these following imaging studies such as MR Venography (MRV) either or multidetector CT venography (CTV). Four patients with DVT and 27 patients with SVT are included. Our control group is the 79 patients who had clinical cerebral venous thrombosis and underwent NCCT, however, eventually proven no cerebral venous thrombosis by either CTV or MRV. Similarly, the patients in control group must have inclusion criteria as present in **Figure 1**.

2.3. Imaging

All patients included in our study had undergone NCCT (n = 110). CT Venography were performed in 90 patients and MR venography were performed in 20 patients.

2.4. Procedure Parameters

All imaging procedures are undergone by the following machines and protocols:

All NCCT were undergone by one of the two 64-slices multidetector CT

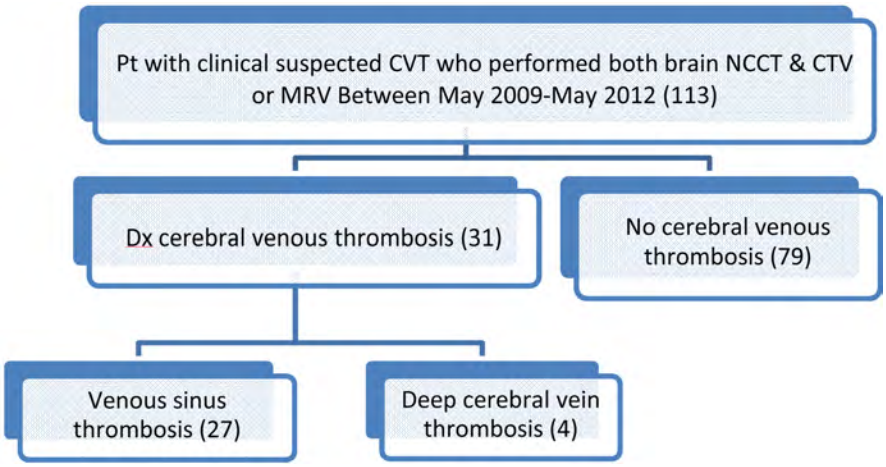

Figure 1. Flow diagram for patient inclusion in this study.

scanners, one from GE (GE heathcare, USA) and another from SIEMENS (Sensation 64; Siemens Medical Solutions, Erlangen, Germany). NCCT protocol used with 120 kV, 300 mAs, collimation 2.5 mm, slices thickness 1.25 mm and 5.0 mm.

All CT Venography were undergone by the same 64-slices multidetector CT scanners. CTV protocol parameters as 120 kV, 120 - 140 mAs; collimation, 4 - 10 mm., 80 - 100 mL contrast medium, injection rate 3 - 5 mL/s and delay 35 s.

All MR Venography were undergone by one of the two 3.0T MR scanner and 1.5 T MR scanner (Inter Achieva, Philips) with protocol parameters as follows:

T1-weighted SE-sequence (TR, 9.5 ms; TE, 8 ms; section thickness, 5 mm; FOV, 230 mm; matrix size, 256).

T2-weighted SE-sequence, (TR, 3000 ms; TE, 90 ms; section thickness, 5 mm; FOV, 230 mm; matrix size, 256).

DWI-sequence with b-values of 0 and 1000, and 3 gradient directions (TR, 2278 ms; TE, 55 ms; section thickness, 5 mm; FOV, 230 mm; matrix size, 128).

Fluid-attenuated inversion recovery (FLAIR) sequence (TR, 11,000 ms; TE, 120 ms; section thickness, 5 mm; FOV, 230 mm; matrix size, 256).

Contrast enhanced MR venography (TR, 9.5 ms; TE, 8 ms; flip angle, 30˚; section thickness, 5 mm; FOV, 230 mm; matrix size, 256).

2.5. Image Review

All of patients' imaging modalities study were interpreted by two neuroradiologists independently without available patients' clinical information and final diagnosis. Reading on PACS workstation in department of Radiology and recorded imaging informations in case record forms.

Recorded imaging data of the NCCT scan are the presence or absence of hyperdense sign (cord sign) in all sinus venous and cortical vein, hyperdense sign (attenuated vein sign) in deep vein. Presence or absence of brain edema, hemorrhagic infarct were also recorded. Then, the readers had to make conclusion for the diagnosis of SVT or DVT. After completing all NCCT interpreta-

tions, the disconcordance cases are in consensus reading of the two readers with obtaining other imaging modalities such as CTV or MRV as well as clinical data of those cases.

2.6. Statistical Analysis

The statistical analysis of our recorded images data was performed by using of standard software (Excel and Access; Microsoft, STATA). The sensitivity and specificity of NCCT in the diagnosis of SVT and DVT as well as 95% confidence intervals were analyzed. Interobserver agreement of the hyperdense sign in sinuses or deep veins were analyzed with multirater values were calculated as described in the literature. The values can range from −1.0 to 1.0, with −1.0 indicating perfect disagreement below chance, 0.0 indicating agreement equal to chance, and 1.0 indicating perfect agreement above chance.

2.7. Results

Four patients with DVT (all are women; age range from 34 to 46 years old; mean age, 43.7 years old). 27 patients with SVT (16 women, 11 men; age range 13 to 72 years old; mean age 42.2 years old. Most common of the clinical manifestations in our patients who were diagnosed as cerebral venous thrombosis were headache (n = 16), seizure (n = 8), paresis of variable degree (n = 7), alteration of consciousness (n = 2), aphasia (n = 2), paresthesia, diplopia, hemianopia, and behavioral change (n = 1 each) present in Table 1. Average time interval from earliest symptoms to diagnosis of cerebral venous thrombosis was 18.43 days.

No significant difference of the mean age of the patients with final diagnosis cerebral venous thrombosis and control group was 41.19 years and 35.09 years, (p value = 0.122) as respectively. No significant difference of the mean hematocrit level of each group was 32.31 and 30.97, (p value = 0.643) as respectively present in Table 2.

Table 1. Clinical symptoms in patient with cerebral vein thrombosis.

Symptoms	Prevalence
headache	51.6% (n = 16)
seizure	25.8% (n = 8)
paresis of variable degree	22.5% (n = 7)
alteration of consciousness	6.4% (n = 2)
aphasia	6.4% (n = 2)
paresthesia, diplopia, hemianopia, behavioral change	(n = 1 each)

Table 2. Mean age and hematocrit level of the included patients.

	Control gr	CVT pos	P value
Mean age	35.09	41.19	0.122
Mean HCt	30.97	32.31	0.643

90 out of 110 patients that have CTV as gold standard, 19 patients were sinovenous thrombosis, 3 patients were deep venous thrombosis and 68 patients were control group. Another 20 patients who have MRV as gold standard, 8 patients were sinovenous thrombosis, 1 patient was deep vein thrombosis and 11 patients were control group. Each reader had reviewed 220 imaging studies evaluation of total sinus venous and deep veins of 3300 venous structures. The result of interpretation in the patients with clinical cerebral venous thrombosis are presented in Tables 3-6.

Our study encountered the hyperattenuating sign in sinus venous structures and deep veins as follows: superior sagittal sinus (SSS) in 48%, the right transverse sinuses (TS) 33.3%, the left transverse sinuses (TS) 36.4%, the right sigmoid sinuses in 16.7%, left sigmoid sinus in 28.6%, cortical vein 81.8%, while the ISS showed no positive case, and the total deep veins thrombosis found in small

Table 3. Result from reading cerebral venous sinus and superficial cerebral vein by 2 readers.

Sinus	Blinded	Consensus reading				Interobserver agreement	Sensitivity (95% CI)	Specitivity (95% CI)
	Cord sign	tp	fp	tn	fn			
SSS	27	22	5	165	28	0.62	44	97
ISS	0	0	0	220	0	-	-	-
LTS	9	7	2	196	15	0.65	31.8	98.9
RTS	12	7	5	181	17	0.47	29.1	97.3
LSS	5	4	1	205	10	0.79	28.6	99.5
RSS	4	2	2	206	10	0.49	16.7	99.0
cortical	18	17	1	195	7	0.51	70.8	99.5
total	75	59	16	1368	87	0.59	40.4	98.84

Table 4. Result from reading deep cerebral veins by 2 readers.

Deep vein	Blinded	Consensus reading				Interobserver agreement	Sensitivity (95% CI)	Specitivity (95% CI)
	Dense vein sign	tp	fp	tn	fn			
SS	2	2	0	214	4	1.00	33.3	100
LICV	2	2	0	218	0	1.00	50	100
RICV	2	2	0	218	0	1.00	50	100
VG	2	2	0	218	0	1.00	100	100
LBVR	0	0	0	220	0	-	-	-
RBVR	0	0	0	220	0	-	-	-
LTSV	2	2	0	218	0	0	50	100
RTSV	2	2	0	218	0	0	50	100
total	12	12	0	1744	4	1.0	75	100

Table 5. Result of consensus reading cerebral venous sinus and superficial cerebral vein.

Sinus	Blinded	Consensus Reading				Sensitivity (95% CI)	Specificity (95% CI)
	Cord sign	TP	FP	TN	FN		
SSS	12	12	0	85	13	48 (27.8 - 68.7)	100 (95.8 - 100)
ISS	0	-	0	110	-	-	-
LTS	4	4	0	99	7	36.4 (10.9 - 69.2)	100 (96.3 - 100)
RTS	4	4	0	98	8	33.3 (9.9 - 65.1)	100 (96.3 - 100)
LSS	2	2	0	103	5	28.6 (3.6 - 71)	100 (96.3 - 100)
RSS	1	1	0	104	5	16.7 (0.4 - 64.1)	100 (96.5 - 100)
Cortical	9	9	1	97	3	81.8 (42.8 - 94.5)	98.9 (96.3 - 100)
Total	32	32	2	695	41	43.8	99.7 (95.9 - 100)

Table 6. Result of consensus reading deep cerebral vein.

Deep vein	Blinded	Consensus reading				Sensitivity (95% CI)	Specitivity (95% CI)
	Dense vein sign	tp	fp	tn	fn		
SS	1	1	0	107	2	50	100
LICV	1	1	0	109	0	100	100
RICV	1	1	0	109	0	100	100
VG	1	1	0	109	0	100	100
LBVR	0	0	0	110	0	-	-
RBVR	0	0	0	110	0	-	-
LTSV	1	1	0	109	0	100	100
RTSV	1	1	0	109	0	100	100
total	6	6	0	872	2	75 (47.8 - 95.7)	100 (96.7 - 100)

number, albeit the sensitivity for overall DVT was about 75% (Table 5 and Table 6) (Figure 2). After the consensus reading, there were 2 false-negative diagnoses of DVT, 41 false-negative diagnosed of SVT, and 1 false-positive diagnoses in the SVT group. Therefore, the sensitivity of NCCT in detection hyperattenuating vein sign for the deep vein thrombosis were 50% and the specificity was 100% whereas the sensitivity and specificity of NCCT in detection the hyperdense sign (cord sign) for the diagnosis of SVT were 43.8% and 99.7% respectively.

Summary of result in interpretation sinus venous thrombosis and deep vein thrombosis as well as false positive and false negative cases are presented in Table 3 and Table 4. The sensitivity and specificity of attenuated vein sign in patients with DVT were 75% and 100% whereas the sensitivity and specificity of cord sign in patients with SVT were 43.8% and 99.7% as respectively. The interobserver agreement of attenuated vein sign was 0.59 (range 0.47 - 0.79; Table 3)

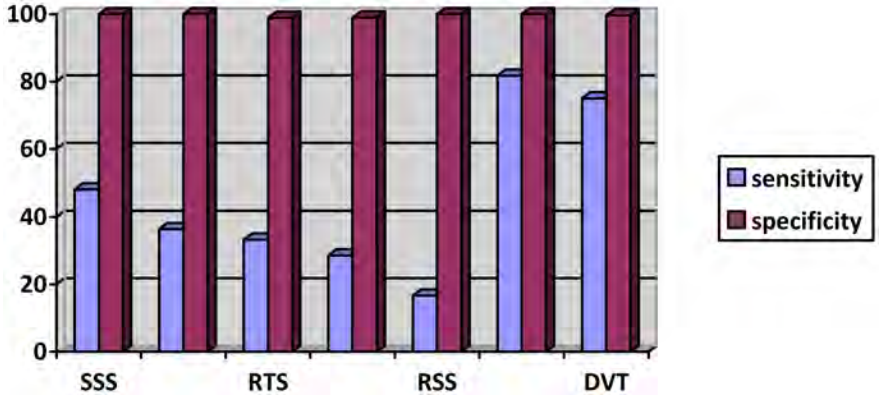

Figure 2. Summary sensitivity and specificity of NCCT brain in diagnosis cerebral venous thrombosis (SSS = superior sagittal sinus, LTS = left transverse sinus, RTS = right transverse sinus, LSS = left sigmoid sinus, RSS = right sigmoid sinus, CV = cortical veins, DVT = deep cerebral vein thrombosis).

and 1.0 for the cord sign (**Table 4**). NCCT detected intracerebral edema was found in noncontrol group 13 cases (41.9%), infarction 8 cases (25.8%), and hemorrhage or hemorrhagic transformations in 8 cases (25.8%).

3. Discussion

Early treatments for the patients with CVT have been proved to obtain an excellent clinical results. Thus, early diagnosis is one of the key success factor of the whole process of patients care particularly in the patients with deep cerebral veins thrombosis [12] [13]. Until the present day, there is long duration time from onset of symptoms to diagnosis of CVT. The main reason is the great variability of the clinical manifestation of CVT patients [14] [15]. Sending D-dimer might not be helpful in diagnosis CVT due to high negative predictive value, normal study could not exclusion of the CVT patients, further indicate the importance of imaging [3]. Catherized cerebral angiography had been the gold standard imaging for diagnosis and follow up treatment in previous days. Nowadays, the procedure of direct cerebral angiography may be considered as invasive imaging procedure. Improvement of technology of noninvasive imaging such as MRI and CT imaging have led to good quality in the visualization of vascular structures either arterial or venous sides with high diagnostic value [2] [3] [10] [16] [17]. However, daily clinical practice in most centers, noncontrast CT brain scan is the forefront imaging study for the patients who have nonspecific neurological symptoms particularly in the emergency departments. The reasons for popularity in usage NCCT study are due to short scan time study, no need for patient preparation nor fully cooperation of the patients nor sedative drugs prescription. No risk of intravenously contrast medium reaction, neprotoxitcity. NCCT still provides good resolution image quality for rapidly diagnosis and prompt further treatment. The patients with CVT are in the same conditions. To review NCCT imaging of our patients in all CVT groups and control groups, the two readers interpreted imaging without clinical information of ce-

rebral venous thrombosis. We found that hyperdensity in the cortical veins is the direct CT evidence of detection intraluminal thrombosis in the cortical veins that called "hyperattenuating vein sign" that similar in deep veins system (**Figure 3** and **Figure 4**) is more sensitive CT sign than the detection of thrombus in the dural venous sinus that called "cord sign" (**Figure 5**). The sensitivity of hyperattenuated vein sign for cortical veins and deep vein thrombosis are 70.8% and 75% whereas cord sign is only 43.8%. Even though, the sensitivity of cord sign by NCCT in diagnosis sinus venous thrombosis is too low in the present study, however, is similar to previous studies sensitivity 25% - 56% [10] [15]. We suggest that the negative case by NCCT in the patients suspected cerebral venous thrombosis might not exclude sinus venous thrombosis (SVT). One main reason of low sensitivity NCCT in SVT found in our study is due to intrasinus thrombosis clot density, if it was isodensity to the brain, it is not possible to diagnosis by means of NCCT (**Figure 6** and **Figure 7**). The density of thrombus is directly related to age of thrombus, the isodensity thrombus is not in acute stage but to subacute or chronic stage which might reflect to variety of clinical symptoms and nonspecific, delayed imaging can occur. Our findings are different from previous study by Alsafi A. *et al*. [18] that those cases are in acute stage hyperattenuated (HU > 67) greater than density of the brain, we found some of cases that isodensity blood clot in the sinuses (**Figure 6** and **Figure 7**) which can cause false negative in the study. Limitations of the present study are first, some cases have only thick slice of NCCT (12 cases), which could cause an increasing rate of false negative results, second, nonhomogenously use standard reference imaging either CTV or MRV and third, number of patient

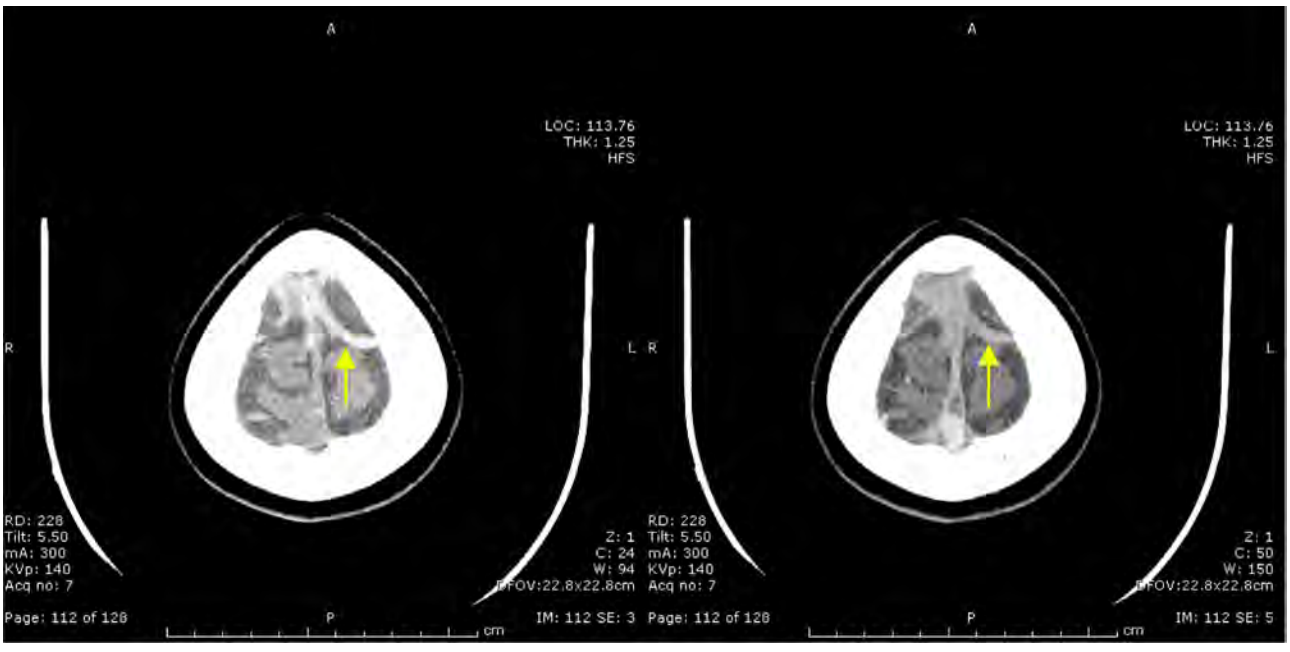

Figure 3. A 14-year-old male patient, systemic lupus erythematosus (SLE) with thrombosis of superior sagittal sinus (SSS) and cortical vein, who presented with headache for 6 months. The NCCT scan shows a true-positive cord sign in the SSS and cortical vein (arrows).

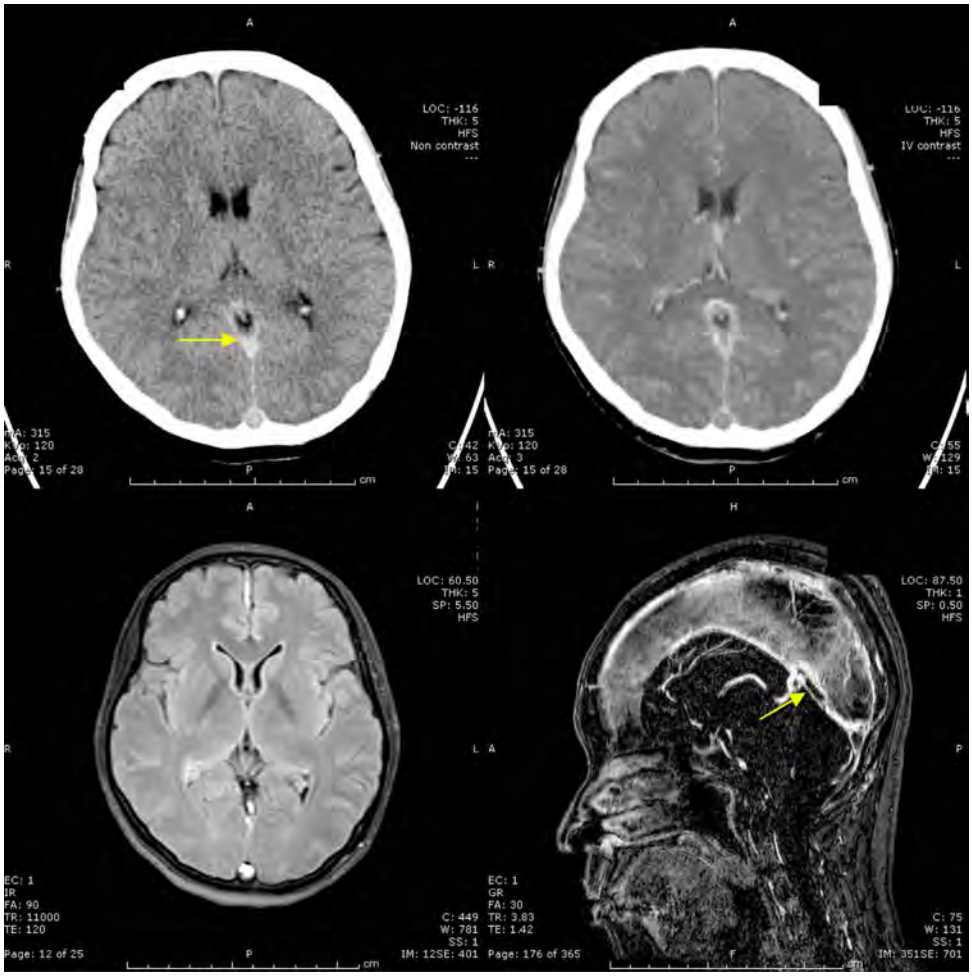

Figure 4. A 44-year-old female patient with venous thrombosis of the SSS, SS, VG. The NCCT scan demonstrates an attenuated vein sign in SS, VG (arrows). The thrombosis is visualized indirectly by demonstration of filling defects in the SS, VG (arrow) on the CTV and MRV.

Figure 5. A 31-year-old female patient in the control group, with sepsis. There are suspected hyperattenuated lesion at SSS and both transverse sinus on NCCT (arrows). This finding was interpreted as a cord sign by all readers, resulting in the false-positive diagnosis of a SVT in this patient. Her Hct was 36.7.

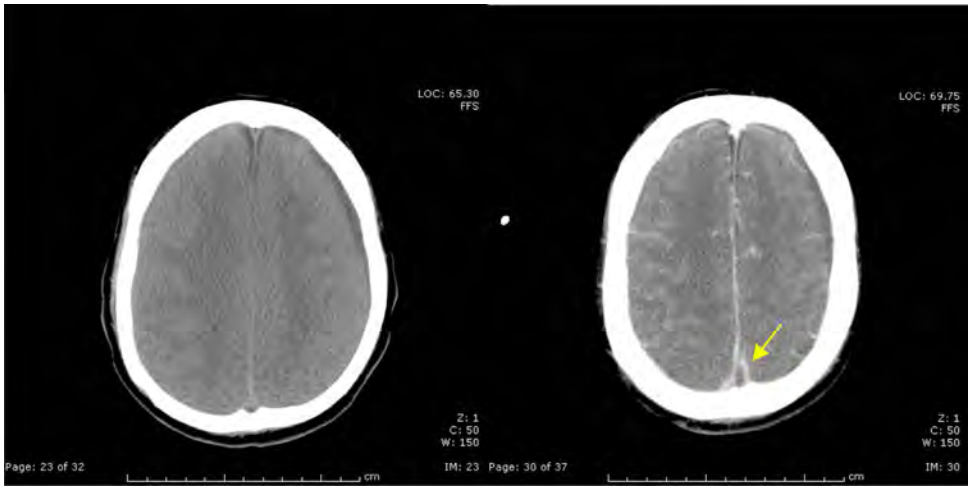

Figure 6. A 39-year old female patient presented with a progressive headache 3 weeks and status epilepticus. The NCCT scan shows no hyperattenuated sign (false-negative). The CTV scan demonstrates contrast-filling defects in posterior part of SSS (arrow), indicating CVT. There is also a chronic subdural collection at left cerebral hemisphere.

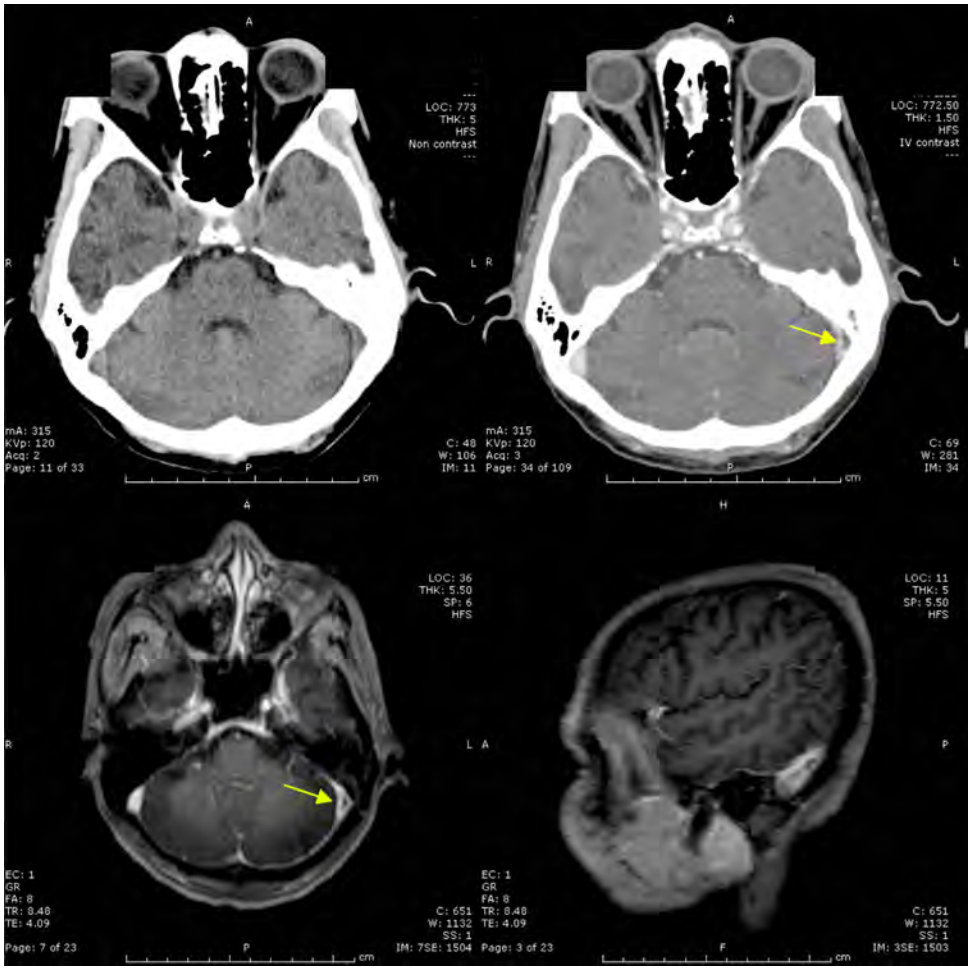

Figure 7. A 47-year old male patient presented with a progressive headache. The NCCT scan shows no hyperattenuated sign (false-negative). MRV demonstrates contrast-filling defects in Left transverse sinus (arrow), indicating CVT.

population is relatively small.

4. Conclusion

NCCT has an impact on the diagnosis of deep cerebral vein thrombosis (DVT) as well as cortical vein thrombosis with high sensitivity and specificity. Absence of hyperattenuating vein signs and parenchymatous changes are less suspicious of deep cerebral vein thrombosis. On contrary, NCCT has poor sensitivity in detection of sinus venous thrombosis (SVT), negative detection suggests to be further investigated by CTV or MRV.

Conflicts of Interest

The authors declare no conflicts of interest regarding the publication of this paper.

References

[1] Karthikeyan, D., Vijay, S., Kumar, T., *et al*. (2004) Cerebral Venous Thrombosis-Spectrum of CT Findings. *Neuroradiology*, **14**, 129-137.

[2] Chiewvit, P., Piyapittayanan, S. and Poungvarin, N. (2011) Cerebral Venous Thrombosis: Diagnostic Dilemma. *Neurology International*, **3**, e13. https://doi.org/10.4081/ni.2011.e13

[3] Ferro, J.M., Canhao, P., Stam, J., *et al*. (2004) Prognosis of Cerebral Vein and Dural sinus Thrombosis: Results of the International Study on Cerebral Vein and Dural sinus Thrombosis (ISCVT). *Stroke*, **35**, 664-670. https://doi.org/10.1161/01.STR.0000117571.76197.26

[4] Raam, R. and Tabatabai, R.R. (2021) Headache in the Emergency Department: Avoiding Misdiagnosis of Dangerous Secondary Causes, an Update. *Emergency Medicine Clinics of North America*, **39**, 67-85. https://doi.org/10.1016/j.emc.2020.09.004

[5] Lee, S.K., Kim, B.S. and TerBrugge, K.G. (2002) Clinical Presentation, Imaging and Treatment of Cerebral Venous Thrombosis (CVT). *Interventional Neuroradiology*, **8**, 5-14. https://doi.org/10.1177/159101990200800102

[6] Saposnik, G., Barinagarrementeria, F., Brown, R.D. and Bushnell, C.D. (2011) Diagnosis and Management of Cerebral Venous Thrombosis. *Stroke*, **42**, 1158-1192. https://doi.org/10.1161/STR.0b013e31820a8364

[7] Idiculla, P.S., Gurula, D., Palanisamy, M., Vijayakumar, R., Dhandapani, S. and Nagarajan, E. (2020) Cerebral Venous Thrombosis: A Comprehensive Review. *European Neurology*, **83**, 369-379. https://doi.org/10.1159/000509802

[8] Stam, J. (2005) Thrombosis of the Cerebral Veins and Sinuses. *The New England Journal of Medicine*, **352**, 1791-1798. https://doi.org/10.1056/NEJMra042354

[9] Rischall, M.A., Boegel, K.H., Palmer, C.S., Knoll, B. and McKinney, A.M. (2016) MDCT Venographic Patterns of Dural Venous Sinus Compromise after Acute Skull Fracture. *American Journal of Roentgenology*, **207**, 852-858. https://doi.org/10.2214/AJR.15.15972

[10] Van Dam, L.F., Van Walderveen, M.A.A., Kroft, L.J.M., Kruyt, N.D. and Wermer, M.J.H. (2020) Current Imaging Modalities for Diagnostic Cerebral Vein Thrombosis—A Critical Review. *Thrombosis Research*, **189**, 132-139.

https://doi.org/10.1016/j.thromres.2020.03.011

[11] Leach, J.L., Fortuna, R.B., Jones, B.V. and Gaskill-Shipley, M.F. (2006) Imaging of Cerebral Venous Thrombosis: Current Techniques, Spectrum of Findings, and Diagnostic Pitfalls. *Radiographic*, **26**, S19-S43. https://doi.org/10.1148/rg.26si055174

[12] Qu, H. and Yang, M. (2013) Early Imaging Characteristics of 62 Cases of Cerebral Venous Sinus Thrombosis. *Experimental and therapeutic Medicine*, **5**, 233-236. https://doi.org/10.3892/etm.2012.796

[13] Caneodo-Antelo, M., Baleato-Gonzalez, S., Mosqueira, A.J., Casas-Martineze, J., *et al*. (2019) Radiologic Clues to Cerebral Venous Thrombosis. *Radiographics*, **39**, 161-1628. https://doi.org/10.1148/rg.2019190015

[14] Luo, Y., Tian, X. and Wang, X. (2018) Diagnosis and Treatment of Cerebral Venous Thrombosis: A Review. *Frontiers in Aging Neuroscience*, **10**, Article No. 2. https://doi.org/10.3389/fnagi.2018.00002

[15] Lee, E.J.Y. (2002) The Empty Delta Sign. *Radiology*, **224**, 788-789. https://doi.org/10.1148/radiol.2243990978

[16] Randolph, J.J. (2005) Free-Marginal Multirater Kappa: An Alternative to Fleiss' Fixedmarginal Multirater Kappa. *Joensuu University Learning and Instruction Symposium*, Joensuu, Finland, 14-15 October 2005, 1-20.

[17] Gaikwad, A.B., Mudalgi, B.A., Patankar, K.B., Patil, J.K. and Ghongade, D.V. (2008) Diagnostic Role of 64-Slice Multidetector Row CT Scan and CT Venogram in Cases of Cerebral Venous Thrombosis. *Emergency Radiology*, **15**, 325-333. https://doi.org/10.1007/s10140-008-0723-4

[18] Alsafi, A., Lakhami, A., Jones, I.C. and Lobotesis, K. (2015) Cerebral Venous Sinus Thrombosis, a Nonenhanced CT Diagnosis? *Radiology Research and Practice*, **2015**, Article ID: 581437. https://doi.org/10.1155/2015/581437

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Melatonin for Pre- and Postoperative Pain and Anxiety: A Cancelled Clinical Trial

Mikkel Zola Andersen, Bennedikte Kollerup Madsen, Dennis Zetner, Camilla Christophersen, Sofie Skovbo Jensen, Jacob Rosenberg

Center for Perioperative Optimization, Department of Surgery, Herlev and Gentofte Hospitals, University of Copenhagen, Herlev, Denmark
Email: mikkelzola@gmail.com

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Abstract

Designing and completing clinical intervention trials can be challenging. Many aspects must be considered to ensure that patients who fulfill the inclusion criteria for the intervention are identified and recruited effectively. The aim of this paper was to disseminate the results of a cancelled trial and present unpredictable barriers met underway, so future researchers can learn from these. The trial examined perioperative analgesic and anxiolytic effects of melatonin. It was registered at https://clinicaltrials.gov/ (NCT02386319) and a study protocol was published a priori. Participants were recruited from the plastic surgery ward of a Danish private hospital. The intended sample size of the trial was 72 patients based on power calculations of the outcome measures. During the six-month recruitment period, six patients were included, with only three completing the trial. Unpredictable barriers were poor communication between investigators and facility staff, lack of access to booking and operation schedules at the recruitment facility, the patient group being unwilling to participate, and the timing of recruitment conversations being unsuited as patients often did not have time to talk to the investigators. Too few data were collected to make any meaningful statistical analyses. Our trial was cancelled prematurely because of unpredictable barriers after commencing recruitment. Considering these barriers when designing a clinical trial may help future researchers avoid cancelling trials. Transparency of research is important and even prematurely cancelled trials should publish their findings.

Keywords

Randomized Controlled Trials, Barriers, Recruitment, Willingness to Participate, Inclusion

1. Introduction

Randomized controlled trials are essential for researching effectiveness of medical treatments [1]. However, designing and completing these may include considerable barriers such as ethical, financial, and personnel issues [2]. Especially the recruitment phase has proven challenging for many trials [2] [3] [4]. Part of this may be explained by rigid legislation when conducting trials with patient involvement. For example, many countries must follow the Good Clinical Practice (GCP) guidelines, which ensure ethical and scientific quality of clinical trials by laying a set of rules to protect the rights of human research subjects [5] [6]. However, GCP has previously been criticized for increasing cost, complexity, and duration of research unnecessarily [7] [8].

Melatonin is an endogenous hormone that plays a large part in controlling the circadian rhythm and is used in many countries to treat sleep disturbances. Additionally, when given exogenously, melatonin has documented effects on pain and anxiety [9] [10]. Therefore, we planned to examine the effect of melatonin on perioperative pain and anxiety. In 2020 the clinical trial was cancelled due to several unforeseeable barriers. Therefore, the aim of this paper was to disseminate the few data collected in the trial and reflect upon barriers that resulted in early termination of the trial, so future researchers hopefully can succeed better than we did.

2. Methods

We conducted a randomized, double-blinded, placebo-controlled clinical trial measuring the effect of melatonin on pre- and postoperative pain and anxiety in patients undergoing elective breast augmentation. It was registered at clinicaltrials.gov (NCT02386319), and a protocol was published a priori [11]. For detailed methods of the trial, see this protocol. The first part of this study, examining pharmacokinetics of oral melatonin, was published in 2016 [12].

Patients were recruited after an outpatient visit with a plastic surgeon to determine eligibility for surgery. To be eligible for the trial, patients had to be eligible for primary breast augmentation or replacement of existing implants. Patients should take one tablet of 10 mg melatonin or placebo four times between the evening before the operation and the evening of the operation. Patients were surveyed several times regarding pain, anxiety, and sleep between the morning of the surgery and 24 hours postoperatively. The intended sample size of the trial was 72 patients based on power calculations of the outcome measures. The trial was conducted in cooperation with a Danish private hospital, and all patients for the trial were recruited from the abovementioned hospital's plastic surgery ward.

All required ethical approvals for the trial were obtained before commencing. These include the Capital Region's Committee on Health Research Ethics (protocol number: H-8-2014-016), the Danish Data Protection Agency (journal number: VD-2019-101), and the Danish Medicines Agency (EudraCT number: 2014-003789-25). The trial was registered at https://clinicaltrials.gov/ (NCT02386319)

before commencing. Informed consent from study participants was obtained both written and verbally.

3. Results

Despite rather massive manpower, only six patients were included between December 2019 and May 2020, of which two completed the trial as intended (Figure 1). In total, 34 patients were screened for inclusion. Of the 28 that were not included in the trial, half (13/28) either did not have time to hear about the trial or left the hospital before researchers could contact them after their outpatient visit. Six patients did not wish to participate after hearing about the trial. Five patients agreed to contact the investigators after considerations. Three of these

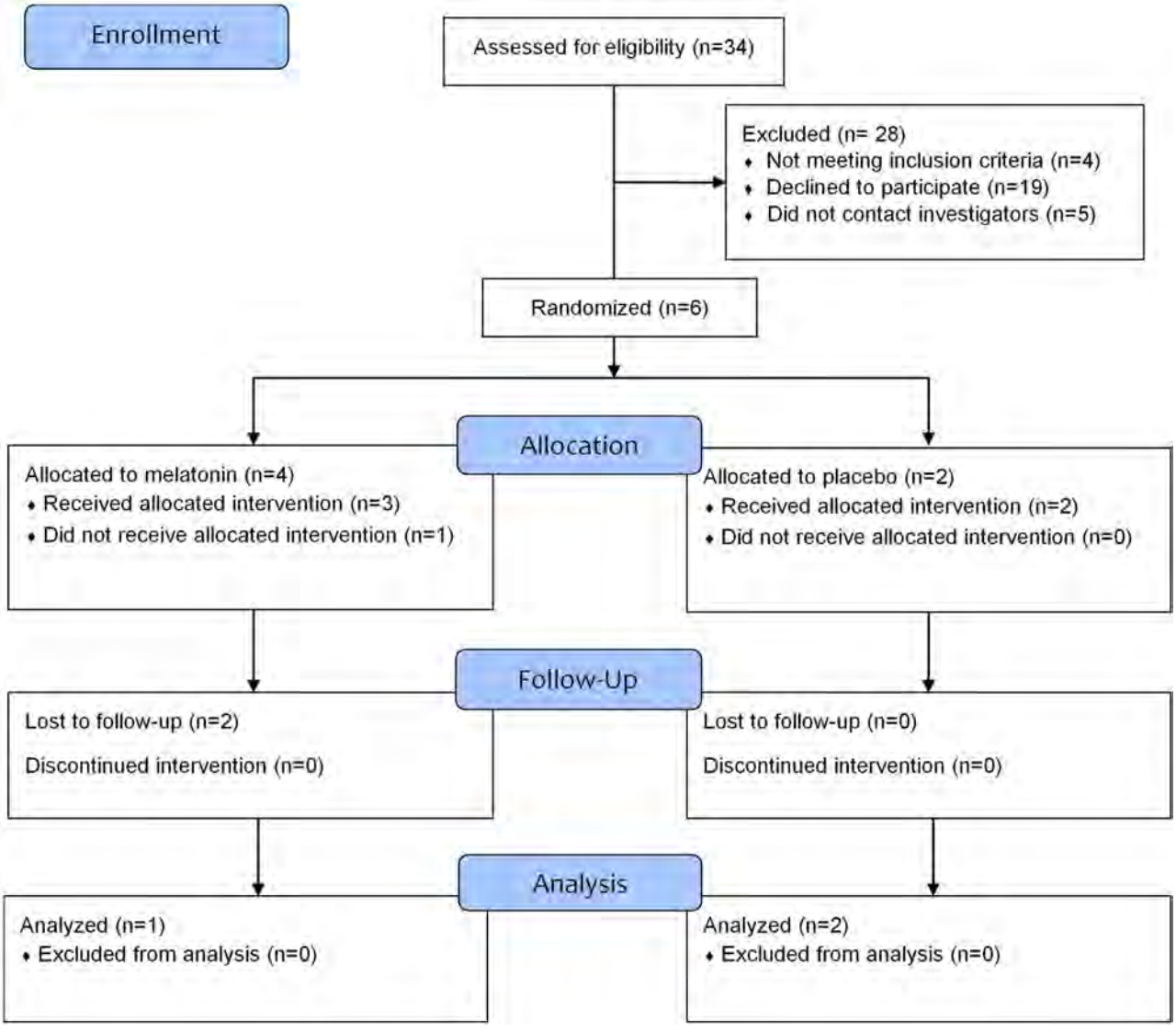

Figure 1. Flow chart of inclusion of patients. One patient did not receive the intervention because she was operated on another day than planned without the investigators' knowledge. Two patients were lost to follow-up as they were operated before planned time, so they did not receive their survey in time. The one patient analyzed in the melatonin group consequently answered surveys 1 - 2 hours after intended.

five wished to consider if they were to have the operation, while two wished to consider if they wanted to participate in the trial. None of the five patients contacted the investigators. Two patients decided against breast augmentation and thus did not meet the inclusion criterion, and two patients were excluded based on the exclusion criteria. Of the six included patients, three patients answered all surveys in the trial. However, one of these patients answered all the surveys 1 - 2 hours late, introducing considerable risk of bias to their results. Of the three patients that did not complete the trial, one underwent surgery on a different day without informing the research team, and the remaining two underwent surgery on the correct day but earlier than planned, again without informing the research team. This resulted in the patients not receiving their surveys on time. Thus, in total, two (6%) of 34 screened patients completed the trial as intended.

We estimated 150 operations yearly based on information provided by the hospital. Despite this, we only encountered 34 patients in six months. This was partly due to poor communication with the ward staff, resulting in the investigators not being contacted when patients were re-booked or made an appointment on short notice, which could happen daily. With the rate of inclusion at the time of cancellation, the trial was set to last 10 years from the beginning of inclusion. Therefore, we chose to terminate the trial as this would prove too costly.

As major barriers for recruitment in our trial, we highlight the time of recruitment conversation, the recruitment demographic, and the recruitment facility (**Figure 2**). Firstly, patients were approached for inclusion following a non-obligating outpatient visit with a plastic surgeon to determine if the patient was a candidate for operation. Some declined to participate in the trial, as they did not know if they would have the surgery. Patients were not warned at the time of booking that researchers would contact them upon arrival at the hospital, as it was not logistically possible, and some did not have time to hear about the trial when approached by investigators. Many left the hospital despite being told at the time of arrival that the investigators would like to talk with them after the plastic surgeon. Secondly, the demographic of young females going for self-payed, cosmetic, one-day surgery in a country with otherwise free public healthcare proved challenging. Several eligible patients were not interested in participating, and others that agreed to contact the investigators did not do so. Thirdly, coming from another institution none of the investigators had any familiarity or connections with the staff when inclusion began, making cooperation more difficult. Additionally, the investigators did not have access to electronic health records in this hospital and to get permission would require several complex and time-consuming applications with ethical and data committees probably lasting up to a year. Therefore, the investigators could not see the actual bookings, which made regular staff involvement necessary. Furthermore, the trial's contact person from the ward was on unexpected leave throughout the inclusion period due to personal matters. This unlucky circumstance made

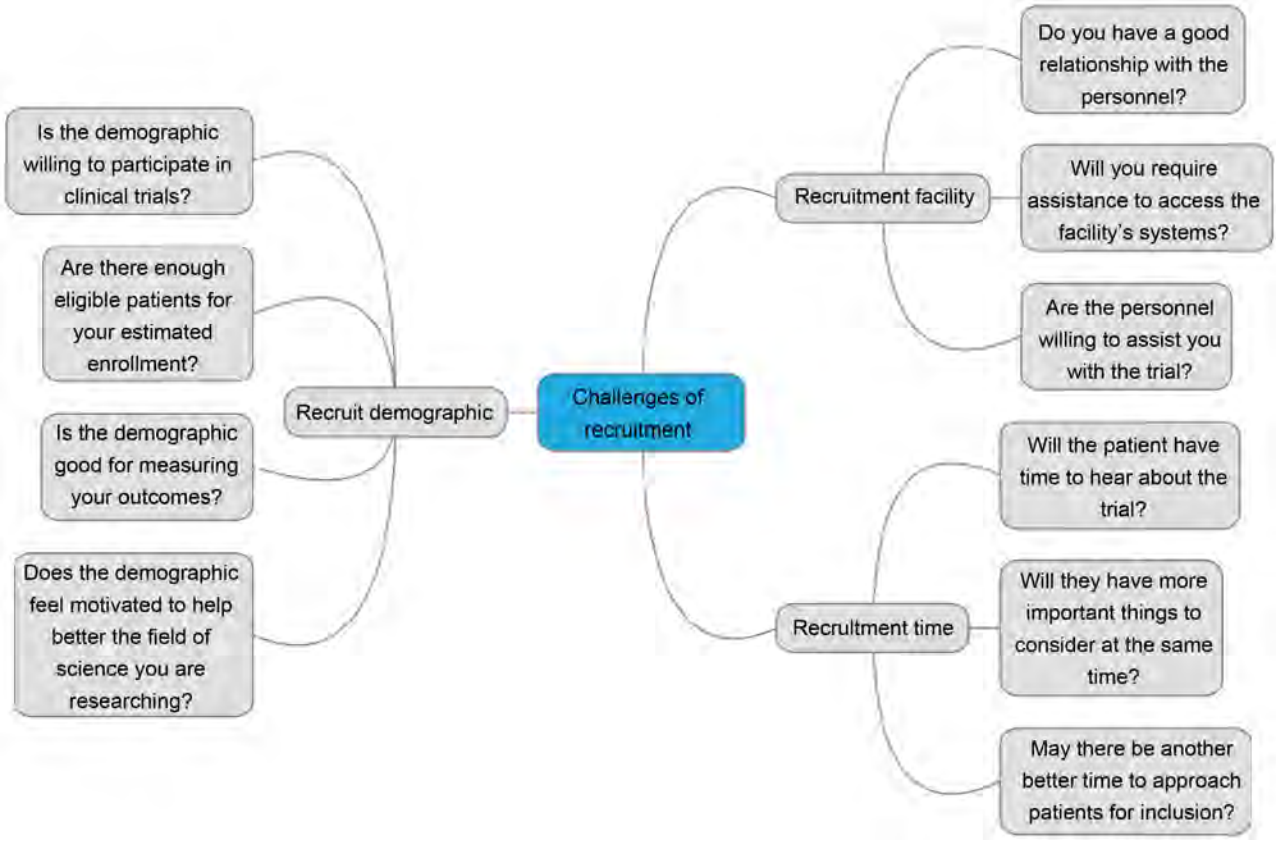

Figure 2. Barriers to consider before starting a randomized clinical trial.

communication at the hospital even more challenging.

The data collected from the six included patients are displayed in **Table 1**. These were too few to make any meaningful analyses but are reported to offer transparency.

This clinical trial was cancelled early owing to unpredictable barriers including: the timing of recruitment conversations being unsuited, as patients often did not have time to talk to the investigators; the patient group being unwilling to participate; poor communication between investigators and facility staff; and lack of access to electronic health records at the recruitment facility. The data collected were too few to make meaningful analyses.

During the six months of inclusion only six patients agreed to participate, of which two patients completed the trial as intended. With the rate of inclusion at the time of trial cancellation, inclusion was set to last 10 years. As randomized controlled trials are costly, and since many other factors around patient care may change in 10 years, it was not possible to keep the trial going for this long, thus, we decided to cancel the trial.

Regarding the time of recruitment conversation, it was not possible to recruit at a better time, as patients would not visit the hospital between attempted recruitment and the first dose of trial drug. Therefore, we had to give the patients the trial drug to bring home after inclusion. According to GCP regulations all

Table 1. Results from the three patients that completed the trial. ID 5 did not take the study medicine and thus had no results to report.

Characteristic	ID 1	ID 2	ID 3*	ID 4**	ID 6*
Intervention	Placebo	Placebo	Melatonin	Melatonin	Melatonin
Age	22	25	21	52	51
Preoperative visit					
VAS pain (rest)	0	0	0	0	1
VAS pain (movement)	0	0	0	0	0
VAS anxiety	3	0	50	0	4
State anxiety	35	25	32	27	24
Trait anxiety	42	29	45	31	32
Morning of surgery					
KSS	3	7	3	1	3
Hours of sleep	7	5.5	6.5	4	5
Quality of sleep	25	27	11	18	70
Fatigue	3	6	3	1	7
Wellbeing	25	31	1	100	50
60 min before surgery					
VAS anxiety	21	18	-	2	-
State anxiety	33	31	-	43	-
Intraoperative					
Remifentanil usage (μg)	40	38	-	25	-
Propofol usage (mL)	62	238	-	41	-
1 hour postoperative					
VAS pain (rest)	56	62	-	23	-
VAS pain (movement)	67	80	-	77	-
VAS anxiety	13	0	-	0	-
2 hours postoperative					
VAS pain (rest)	66	18	-	38	-
VAS pain (movement)	77	36	-	77	-
VAS anxiety	21	0	-	6	-
4 hours postoperative					
VAS pain (rest)	72	8	-	0	-
VAS pain (movement)	93	27	-	95	-
VAS anxiety	32	0	-	0	-
State anxiety	36	27	-	31	-
6 hours postoperative					
VAS pain (rest)	35	11	-	18	-
VAS pain (movement)	35	28	-	20	-
Opioid usage (oxycodon mg)	10	10	-	17.5	-

Continued

8 hours postoperative					
VAS pain (rest)	19	6	-	1	-
VAS pain (movement)	23	16	-	22	-
Time of release from hospital					
Time in recovery (hours)	3	1.5	-	2	-
Total time in hospital (hours)	3	7	-	2	-
Morning after surgery					
KSS	7	3	-	2	-
Hours of sleep	6	6.5	-	5	-
Quality of sleep	71	28	-	84	-
Fatigue	6	4	-	1	-
Wellbeing	63	30	-	15	-
24 hours postoperative					
Opioid usage (mg)	2.5	40	-	45	-
VAS pain (rest)	32	15	-	0	-
VAS pain (movement)	74	30	-	30	-

VAS: Visual Analog Scale (1 - 100); STAI: State-Trait Anxiety Inventory. Scale from 20 - 80 each for state anxiety and trait anxiety, with higher values indicating more anxiety [13]. KSS: Karolinska Sleepiness Scale. Scale from 1 - 9 with higher values indicating more sleepiness [14]. Quality of sleep: Scale from 1 - 100 with higher values indicating lower quality of sleep. Fatigue: Scale from 1 - 10 with higher values indicating more fatigue. Wellbeing: Scale from 1 - 100 with higher values indicating less wellbeing. *: Did not complete the trial; **: Answered surveys 1 - 2 hours after intended.

aspects of trial drug storage should be documented sufficiently [6]. As the patients could not document storage of the trial drug in their home, we were unable to retrieve study medicine if the patient decided to drop out of the trial. This was despite the only storage requirement for the drug being storage below 25˚C, which would be easy for the patients to comply with, even when storing the medicine in their homes. As we did not have much more medicine than needed to meet power calculations, losing medicine to patients dropping out was costly. If this GCP regulation did not exist it would be more manageable if patients dropped out, as we could retrieve the medicine if the patient had not taken the first dosage. An operation could also be chosen that required a follow-up visit to the hospital after booking the operation and use this time for inclusion, e.g. an appointment with an anesthesiologist prior to surgery. This might result in patients being more decided on operation at the time of recruitment conversation. Alternatively, an operation where patients are very certain, prior to their appointment with the doctor, that they will indeed have the surgery could be chosen, e.g. elective inguinal hernia repair. Attempts to inform the patient that they would be asked by an investigator to participate in a clinical trial at the time of outpatient visit booking may have improved inclusion rates. In general, howev-

er, it is uncertain which information must be presented to patients to improve recruitment [15].

The operation chosen resulted in the demographic for inclusion and while the demographic was good for measuring the outcomes, willingness to participate and compliance was not optimal. It has been argued that recruitment of surgical patients for clinical trials is unpredictable [16]. Patients paying for their own operation in a country with free public health care may be less willing to participate in clinical trials, if this group of patients does not feel obligated to contribute to research, as they have paid for their own treatment and are not offered anything in return. Monetary incentives have been shown to be successful in recruiting and maintaining patients in clinical trials [15] [17] [18] [19] [20] [21]. However, in Denmark, where this trial was conducted, monetary incentives for patients are not allowed. Previous trials have had difficulties recruiting patients to trials of self-payment versus funded treatment [22]. This may be interesting to examine qualitatively in the future. Thus, another type of surgery, perhaps without patient payment involved, should probably have been chosen.

Regarding the recruitment facility, it is important to ensure a good relationship with the ward staff to ease communication. In retrospect, as we were not contacted with updates of patient bookings, we should have contacted the staff more often for information. However, it is also important not to annoy the staff with constant contact. If possible, using a hospital where one of the investigators works regularly might improve communication and recruitment. Yet, for our trial, the operation chosen did not allow this. We had not acquired permission to access the electronic health records at the hospital before starting inclusion. This should be a consideration when planning a trial. Had we done so, it would have allowed the investigators to follow up on patient bookings and operations without involving the staff. Access to electronic systems may potentially make clinical research more effective [23], but ethical issues regarding security must also be considered [24]. Alternatively, involving the staff at the hospital in the recruitment process to a higher degree may improve communication and recruitment, but this may be difficult when there are no incentives involved.

4. Conclusion

Many barriers may arise in designing and conducting clinical trials, and some of these may present themselves late in the process. Our trial was cancelled due to unpredictable barriers during the recruitment phase of the trial. Major barriers were related to poor communication between investigators and ward staff, lack of access to electronic health records, the patient group being unwilling to participate, and the recruitment time being unsuited as patients often did not have time to talk to the investigators. These barriers should be considered by future researchers before commencing inclusion. Transparency in research is important, and results of clinical trials should be reported, even if they do not offer sound conclusions. Our trial is one such example.

Conflicts of Interest

The authors declare no conflicts of interest regarding the publication of this paper.

References

[1] Hariton, E. and Locascio, J.J. (2018) Randomised Controlled Trials—The Gold Standard for Effectiveness Research. *BJOG*, **125**, 1716. https://doi.org/10.1111/1471-0528.15199

[2] Varse, F., Janani, L., Moradi, Y., Solaymani-Dodaran, M., Baradaran, H.R. and Rimaz, S. (2019) Challenges in the Design, Conduct, Analysis, and Reporting in Randomized Clinical Trial Studies: A Systematic Review. *Medical Journal of the Islamic Republic of Iran*, **33**, 37. https://doi.org/10.47176/mjiri.33.37

[3] McDonald, A.M., Knight, R.C., Campbell, M.K., Entwistle, V.A., Grant, A.M., Cook, J.A., *et al*. (2009) What Influences Recruitment to Randomised Controlled Trials? A Review of Trials Funded by Two UK Funding Agencies. *Trials*, **7**, 9. https://doi.org/10.1186/1745-6215-7-9

[4] Thoma, A., Farrokhyar, F., McKnight, L. and Bhandari, M. (2010) Practical Tips for Surgical Research: How to Optimize Patient Recruitment. *Canadian Journal of Surgery*, **53**, 205-210.

[5] Vijayananthan, A. and Nawawi, O. (2008) The Importance of Good Clinical Practice Guidelines and Its Role in Clinical Trials. *The Biomedical Imaging and Intervention Journal*, **4**, e5. https://doi.org/10.2349/biij.4.1.e5

[6] Guideline for Good Clinical Practice E6(R2). https://www.ema.europa.eu/en/documents/scientific-guideline/ich-e-6-r2-guideline-good-clinical-practice-step-5_en.pdf

[7] Grimes, D.A., Hubacher, D., Nanda, K., Schulz, K.F., Moher, D. and Altman, D.G. (2005) The Good Clinical Practice Guideline: A Bronze Standard for Clinical Research. *The Lancet*, **366**, 172-174. https://doi.org/10.1016/S0140-6736(05)66875-4

[8] Mentz, R.J., Hernandez, A.F., Berdan, L.G., Rorick, T., O'Brien, E.C., Ibarra, J.C., *et al*. (2016) Good Clinical Practice Guidance and Pragmatic Clinical Trials: Balancing the Best of Both Worlds. *Circulation*, **133**, 872-880. https://doi.org/10.1161/CIRCULATIONAHA.115.019902

[9] Srinivasan, V., Pandi-Perumal, S.R., Spence, D.W., Moscovitch, A., Trakht, I., Brown, G.M., *et al*. (2010) Potential Use of Melatonergic Drugs in Analgesia: Mechanisms of Action. *Brain Research Bulletin*, **81**, 362-371. https://doi.org/10.1016/j.brainresbull.2009.12.001

[10] Ochoa-Sanchez, R., Rainer, Q., Comai, S., Spadoni, G., Bedini, A., Rivara, S., *et al*. (2012) Anxiolytic Effects of the Melatonin MT2 Receptor Partial Agonist UCM765: Comparison with Melatonin and Diazepam. *Progress in Neuro-Psychopharmacology & Biological Psychiatry*, **39**, 318-325. https://doi.org/10.1016/j.pnpbp.2012.07.003

[11] Kollerup Madsen, B., Zetner, D. andersen, L.P.K. and Rosenberg, J. (2019) The Anxiolytic and Analgesic Effects of Melatonin: A Study Protocol for a Randomized, Double-Blind, Placebo-Controlled Study. *Melatonin Research*, **2**, 22-34. https://doi.org/10.32794/mr11250019

[12] Harpsøe, N.G. andersen, L.P.K., Mielke, L.V., Jønsson, B., Jenstrup, M.T., Gögenur, I., *et al*. (2016) Pharmacokinetics of Repeated Melatonin Drug Administrations Prior to and after Surgery. *Clinical Drug Investigation*, **36**, 1045-1050.

https://doi.org/10.1007/s40261-016-0452-5

[13] Spielberger, C.D., Gorsuch, R.L., Lushene, R., Vagg, P.R. and Jacobs, G.A. (1983) Manual for the State-Trait Anxiety Inventory. Consulting Psychologists Press, Palo Alto.

[14] Kaida, K., Takahashi, M., Åkerstedt, T., Nakata, A., Otsuka, Y., Haratani, T., *et al*. (2006) Validation of the Karolinska Sleepiness Scale against Performance and EEG Variables. *Clinical Neurophysiology*, **117**, 1574-1581. https://doi.org/10.1016/j.clinph.2006.03.011

[15] Treweek, S., Pitkethly, M., Cook, J., Fraser, C., Mitchell, E., Sullivan, F., *et al*. (2018) Strategies to Improve Recruitment to Randomised Trials. *Cochrane Database of Systematic Reviews*, **2**, MR000013. https://doi.org/10.1002/14651858.MR000013.pub6

[16] Jack, W.J.L., Chetty, U. and Rodger, A. (1990) Recruitment to a Prospective Breast Conservation Trial: Why Are So Few Patients Randomised? *BMJ*, **301**, 83-85. https://doi.org/10.1136/bmj.301.6743.83

[17] Yu, S., Alper, H.E., Nguyen, A.M., Brackbill, R.M., Turner, L., Walker, D.J., *et al*. (2017) The Effectiveness of a Monetary Incentive Offer on Survey Response Rates and Response Completeness in a Longitudinal Study. *BMC Medical Research Methodology*, **17**, 77. https://doi.org/10.1186/s12874-017-0353-1

[18] Griffin, J.M., Simon, A.B., Hulbert, E., Stevenson, J., Grill, J.P., Noorbaloochi, S., *et al*. (2011) A Comparison of Small Monetary Incentives to Convert Survey Non-Respondents: A Randomized Control Trial. *BMC Medical Research Methodology*, **11**, 81. https://doi.org/10.1186/1471-2288-11-81

[19] Agarwal, A., Raad, D., Kairouz, V., Fudyma, J., Curtis, A.B., Schünemann, H.J., *et al*. (2016) The Effect of a Monetary Incentive for Administrative Assistants on the Survey Response Rate: A Randomized Controlled Trial. *BMC Medical Research Methodology*, **16**, 94. https://doi.org/10.1186/s12874-016-0201-8

[20] Pit, S.W., Vo, T. and Pyakurel, S. (2014) The Effectiveness of Recruitment Strategies on General Practitioner's Survey Response Rates—A Systematic Review. *BMC Medical Research Methodology*, **14**, 76. https://doi.org/10.1186/1471-2288-14-76

[21] Hardy, P., Bell, J.L., Brocklehurst, P., Bick, D., Briley, A., Juszczak, E., *et al*. (2016) Evaluation of the Effects of an Offer of a Monetary Incentive on the Rate of Questionnaire Return during Follow-Up of a Clinical Trial: A Randomised Study within a Trial. *BMC Medical Research Methodology*, **16**, 82. https://doi.org/10.1186/s12874-016-0180-9

[22] Tudor, K., Jebb, S.A., Manoharan, I. and Aveyard, P. (2020) Brief Interventions for Obesity When Patients Are Asked to Pay for Weight Loss Treatment: An Observational Study in Primary Care with an Embedded Randomised Trial. *British Journal of General Practice*, **70**, e348-e355. https://doi.org/10.3399/bjgp20X708797

[23] Coorevits, P., Sundgren, M., Klein, G.O., Bahr, A., Claerhout, B., Daniel, C., *et al*. (2013) Electronic Health Records: New Opportunities for Clinical Research. *Journal of Internal Medicine*, **274**, 547-560. https://doi.org/10.1111/joim.12119

[24] Powell, J. and Buchan, I. (2005) Electronic Health Records Should Support Clinical Research. *Journal of Medical Internet Research*, **7**, e4. https://doi.org/10.2196/jmir.7.1.e4

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A Systematic Review on Chemical Constituents of Suanzaoren Decoction, a Traditional Chinese Medicine Prescription

Wenchao Gu*, Tianyuan Ye*, Liangkun Zhang, Yong Yang, Dongmei Qi, Xiaorui Cheng#, Xin Wang#

Shandong University of Traditional Chinese Medicine, Jinan, China
Email: #cxr916@163.com, #xinw0422@126.com

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Abstract

Traditional Chinese Medicine prescription Suanzaoren decoction (SZRD) is composed of Ziziphi Spinosae Semen, Chuanxiong Rhizoma, Anemarrhenae rhizoma, Poria and Licorice. It was used to treat central nervous system diseases such as insomnia and anxiety for thousands of years. This paper aims to systematically understand varieties and quantities of compounds and clarify chemical components of SZRD, subsequently to further provide the reference for phytochemistry and pharmacology researches of SZRD. Our results showed that SZRD contained 145 components, including flavonoids, triterpenoids, steroids, coumarins, phthalides, and volatile oils, etc., while five-single herbs contain 1104 components. Only in terms of compound number, there were 80 common components in SZRD and its five herbs, which accounted for 6.8% of total compounds in all 5 herbs and 55.2% of compounds in SZRD. The components of SZRD were not simply the sum of one in every single herb. It is necessary to perform parallel studies among SZRD and its herbs. This review discussed the problems that existed in the chemical research of SZRD and pointed out the direction for its further research.

Keywords

Traditional Chinese Medicine, Suanzaoren Decoction, Chemical Ingredient

1. Introduction

Suanzaoren decoction (SZRD), a classic Traditional Chinese Medicine (TCM) prescription, was first described in the book "*Jin Gui Yao Lue*" by Zhongjing

*Co-first authors, these two authors contributed equally to this work.
#Corresponding authors.

Zhang in Han dynasty. SZRD is consists of five herbs, Suanzaoren (Ziziphi Spinosae Semen, *Ziziphus jujuba Mill. var. spinosa* (*Bunge*) *Hu ex H.F. Chou*), Chuanxiong (Chuanxiong Rhizoma, *Ligusticum chuanxiong Hort.*), Zhimu (Anemarrhenae rhizoma, *Anemarrhena asphodeloides Bge.*), Fuling (Poria, *Poria cocos* (*Schw.*) *Wolf*) and Gancao (Licorice, *Glycyrrhiza uralensis Fisch.*, *Glycyrrhiza inflata Bat. or Glycyrrhiza glabra L.*) at a ratio of 15:6:6:6:3. In the clinic of TCM, SZRD is the most common herbal formula prescribed by TCM doctors to treat central nervous system diseases such as insomnia [1] [2] [3], secondary insomnia [4] [5] [6] [7], anxiety [8], depression [9], menopausal syndrome [10] and other diseases [11] [12]. Clinical researches showed that the combination of SZRD and other drugs, including TCM and chemicals, can improve insomnia [13] [14] [15] [16], anxiety [16] [17] [18], depression [19] [20] and other diseases [21], enhance the efficiency of treatment, and reduce adverse reactions. Besides, SZRD has pharmacological characteristics of immune protective and sedative effects [22].

The clinical therapeutic effect and pharmacological function of SZRD originate in its chemical composition. A vast lot of chemical components in SZRD have been isolated and identified using high-performance liquid chromatography (HPLC) and mass spectrometry (MS) technology. In this paper, we investigated and analyzed chemical ingredients of SZRD and its five-single herbs on basis of literatures in the last 5 years and calculated the percentage based on compounds number of a certain class in the total number of compounds. We also focused on similarities and differences of ingredients between SZRD and its herbs.

2. Chemical Constituents of Five Herbs in Suanzaoren Decoction

2.1. Suanzaoren (Ziziphi Spinosae Semen, *Ziziphus jujuba* Mill. var. *spinosa* (Bunge) Hu ex H.F. Chou)

We used "Suanzaoren", "Zaoren", "Ziziphi Spinosae Semen", "Jujube", "*Ziziphus jujuba Mill. var. spinosa* (*Bunge*) *Hu ex* H. F. Chou" as key words to search chemical components of Ziziphi Spinosae Semen. We mainly focused on the literatures of the past five years, and tried to get more ingredients at the same time. Results showed mounts of literatures on ingredients of Ziziphi Spinosae Semen have been reported until now. The 36 chemical components in standard decoction of Ziziphi Spinosae Semen are isolated by using UPLC-Q/TOF-MS and UPLC-PDA system, including nucleosides, phenolic acids, alkaloids and flavonoids [23]. The 109 components are identified in Ziziphi Spinosae Semen by UPLC-Q/TOF-MS and principal component analysis (PCA), including 66 flavonoids, 15 triterpenoid saponin, 19 alkaloids, 8 terpenoid acids and 1 phenolic acid [24]. Research on components in crude and parched Ziziphi Spinosae Semen shows that 40 chemical ingredients (flavonoids, saponins, alkaloids and triterpenoids, etc.) are changed during stir-frying process and 19 key markers

can contribute to classification of crude and parched Ziziphi Spinosae Semen [25]. The sedative and hypnotic effects of Ziziphi Spinosae Semen were significantly enhanced after processing. These researches provided a rapid and effective approach to monitor quality consistency of Ziziphi Spinosae Semen.

We totally collected 175 compounds in Ziziphi Spinosae Semen [23]-[40], which were divided into 8 categories, including flavonoids (62 compounds), alkaloids (28 compounds), amino acids (6 compounds), nucleosides (4 compounds), phenolic acids (4 compounds), triterpenoids (61 compounds), volatile oils (9 compounds) and others (1 compound) (**Table 1**). Ziziphi Spinosae Semen has the highest proportion of flavonoids (36%), followed by triterpenes (35%), and the lowest proportion of phenolic acids (2%) and nucleotides (2%) (**Figure 1(a)**).

2.2. Chuanxiong (Chuanxiong Rhizoma, *Ligusticum chuanxiong Hort.*)

The chemical constituents of Chuanxiong Rhizoma are searched by using "Chuanxiong", "Chuanxiong Rhizoma", "*Ligusticum chuanxiong Hort.*", "*Ligusticum sinense Oliv*" and we focused on the literature of the past five years, including English and Chinese literature. The study indicated that 73 chemical compounds in the essential oil from Chuanxiong Rhizoma were detected and 33 compounds with main component Z-Ligustilide were identified by gas chromatgraphy-mass spectrometry (GC-MS) [41]. The other study showed that 30 chemical components in Chuanxiong Rhizoma were separated and identified by using ultra-performance liquid chromatography with electrospray ionization-time of flight mass spectrometry (UPLC-ESI-TOF/MS) technology, including 20 phthalides, 2 flavonoids, 1 alkaloid and 7 phenolic acids [42]. Research on components of dried rhizome from Chuanxiong Rhizoma showed that 10 phthalide derivatives were isolated by using HPLC technology and spectroscopic analyses and 6 of them were identified as ligusticoside A-F for the first time [43].

Chuanxiong Rhizoma contained 324 compounds, which could be divided into 10 categories [41]-[56], including volatile oils (162 compounds, 50%), phthalides (chemical classification instead of plastic pollution) (85 compounds, 26%), phenolic acids (20 compounds, 6%), polysaccharides (17 compounds, 5%), alkaloids (14 compounds, 4%), flavonoids (6 compounds, 2%), ceramides and cerebrosides (5 compounds, 2%), coumarins (3 compounds, 1%), triterpenoids (4 compounds, 1%) and others (8 compounds, 3%) (**Table 2**, **Figure 1(b)**).

2.3. Zhimu (Anemarrhenae rhizoma, *Anemarrhena asphodeloides Bge.*)

We used "Zhimu", "Anemarrhenae rhizoma", "*Anemarrhena asphodeloides Bge.*", "*Anemarrhena asphodeloides Bunge*" as key words to search chemical components of Anemarrhenae rhizoma, and the literatures were mainly in recent years which contained all the components as much as possible. It's reported that 32 chemical constituents were identified in Anemarrhenae rhizoma, which

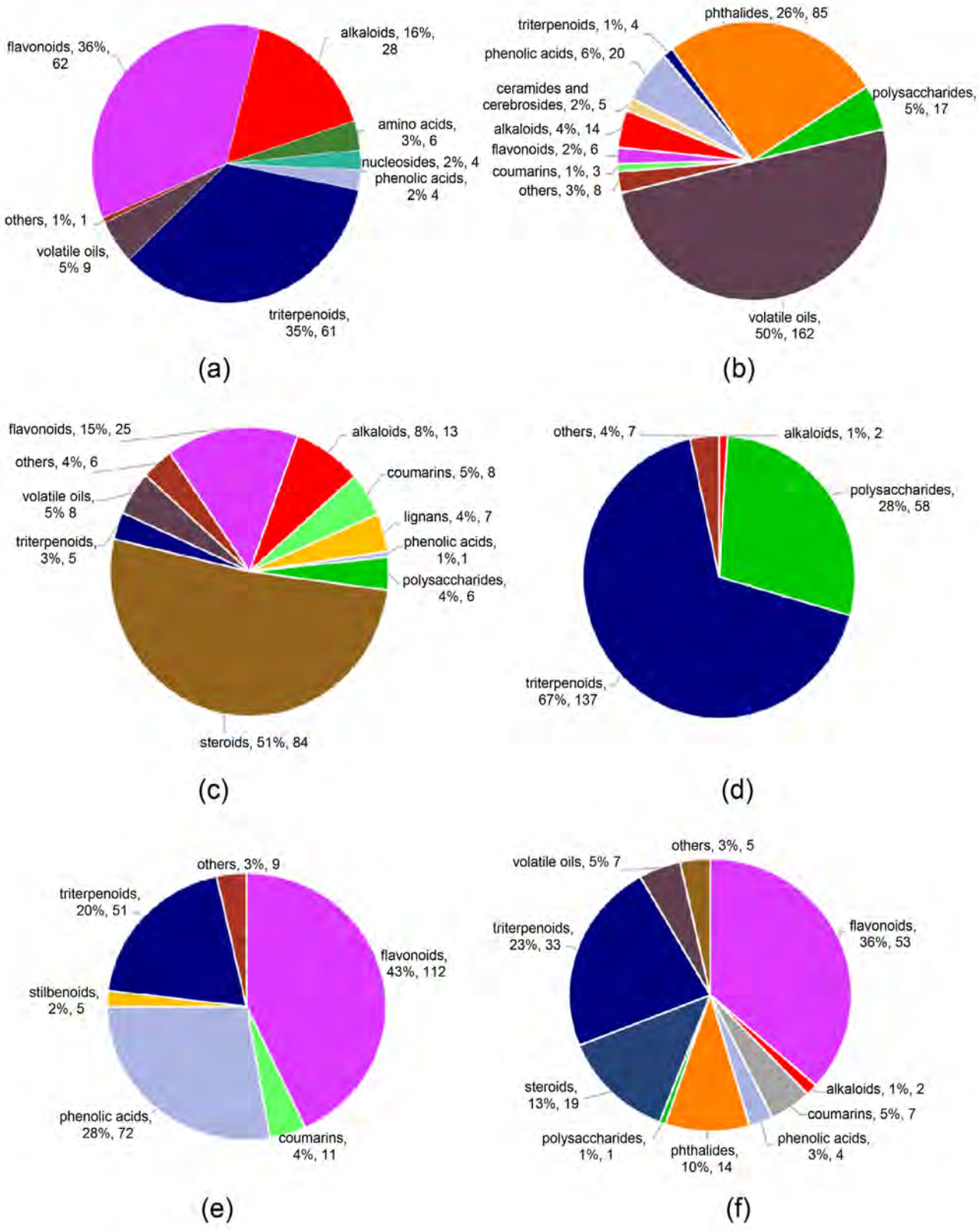

Figure 1. Chemical constituents in suanzaoren decoction and its herbs. (a) Constituents in Ziziphi Spinosae Semen; (b) Constituents in Chuanxiong Rhizoma; (c) Constituents in Anemarrhenae rhizome; (d) Constituents in Poria; (e) Constituents in Licorice; (f) Constituents in SZRD.

Table 1. Chemical constituents in Suanzaoren (Ziziphi Spinosae Semen, *Ziziphus jujuba Mill. var. spinosa* (*Bunge*) *Hu ex* H. F. Chou).

Class	Compound
Triterpenoids	24-hydroxyceanothic acid, 2-o-protocatechuoyl aliphitolic acid, 2α-hydroxypyracrenic acid, 3-ketoursolic acid, 5α,8α-epidioxy-(22e,4r)-ergosta-6,22-dien-3β-ol, acetyl jujuboside B, alhpitolic acid, betulic acid, betulin, betulinic acid, betutin, campesterol, ceanothenic acid, ceanothic acid, epiceanothic acid, jujuboside A, jujuboside A_1, jujuboside A_2, jujuboside B, jujuboside B_1, jujuboside c, jujuboside D, jujuboside E, jujuboside G, jujuboside h, jujuboside I, jujuboside II, jujuboside III, jujuboside IV, lupeol, methyl betulinate, projujuboside B, protojujuboside A, protojujuboside B, protojujuboside B_1, protojujuboside D, protojujuboside H, pseudolaroside B, stigmast-4-en-3-one, ursolic acid, 2α-hydroxyursolic acid, 3-o-cis-p-coumaroyl alphitolic acid, 3-o-cis-p-coumaroyl maslinic acid, 3-o-protocatechuoyl ceanothic acid, 3-o-trans-p-coumaroyl alphitolic acid, 3-o-trans-p-coumaroyl maslinic acid, Azukisaponin II, betulonic acid, cecropiacic acid, colubrinic acid, corosolic acid, hydroxyoleanonic acid lactone, isoceanothic acid, lulutonic acid, maslinic aicd, oleanolic acid, pomonic acid, zizyberanalic acid, zizyphus saponin I, zizyphus saponin II, zizyphus saponin III
Flavonoids	6-hydroxyflavone, 6'''-(-)-phaseoylspinosin, 6'''-(4'''-o-β-d-glucopyranosyl)-vanilloylspinosin, 6'''-(4''''-o-glu)-p-hydroxyben zoylspinosin, 6'''-diferuloylspinosin, 6'''-dihydrophaseoylspinosin, 6'''-ferulylspinosin, 6'''-pcoumaloylspinosin, 6'''-pcoumaroylspinosin, 6'''-phydroxybenzoylspinosin, 6'''-phydroxylbenzoyspinosin, 6'''-sinapoylspinosin, 6'''-vanilloylspinosin, 6"6'''-diferuloylisospinosin, acylated flavuone c-glycoside I, acylated flavuone c-glycoside II, acylated flavuone c-glycoside III, apigenin-6-c-[(6-o-phydroxybenzyol)-(β-d-glucopyranosyl(1→2)(β-d-glucopyranoside), isospinosin, isovitexin-2, isovitexin-2"-o-(6-feruloyl)-glucopyranoside, isovitexin-2"-o-d-glucopyranoside, isovitexin-2"-o-glucopyranoside, isovitexin-2"-o-β-d-glc, isovitexin-2"-o-β-d-glucopyranoside, puerarin, quercetin, rutin, spinorhamnoside, spinosina, vitexin-4"-o-glucoside, zivulgarin, 7-o-(6'''-o-feruloylglucosyl)-isocytisoside, catechin, 4-o-(6'''-o-feruloylglucosyl)-swertisin, 6"-feruloyl-6'''-vanillylspinosin, isoswertisin, ispinosin, kaemperol-3-o-rutinoside, kaempferol 3-o-α-l-rhamnopyranosyl-(1→2)-o-[o-α-l-rhamnopyranosyl-(1→6)]-β-d-glucopyranoside, kaempferol-3-o-β-d-xylopyranosyl-(1→2)-[α-l-rhamnopyranosyl-(1→6)]-β-d-glucopyrano-side), kaempferol-3-rutinoside, 2"-o-glucoylisoswertisin, 6",6'''-diferuloylspinosin, apigenin-6-c-d-glucopyranoside, 2"-b-o-glucopyranosyl swertisin, swertish, isopinosina, epicatechin, 4-(2-methoxy-phenyl)-l-[2-(n-2"-pyridinyl)-p-iodobenza-mido]-ethyl-piperazine, camelliaside B, catechine, nervilifordin J, 6'''-feruloylspinosin, glucosylvitexin, nicotiflorin, saponarin, spinosin, swertisin, isoquercitrin, isovitexin, vicenin II
Alkaloids	amphibine, coclaurine K, lysicamine, nornuciferine I_a, nornuciferine I_b, nuciferine e, sanjoinine A, sanjoinine B, sanjoinine C, sanjoinine D, sanjoinine e, sanjoinine F, sanjoinine G_1, sanjoinine G_2, sanjoinine I_a (nornuciferine), sanjoinine I_b (norisocorydine), zizyphusine, 5-hydroxy-6-methoxynoraporphine, n-methylasimilobine, 6-(2',3'-dihydroxyl-4'-hydroxymethyl-tetrahydro-furan-1'-yl)-cyclopentene[c]pyrrole-1,3-diol, jubanine C, jubanine F, jujube cyclic peptide, juzirine, nummularine B, adenosine, magnoflorine, tryptophan
Amino Acids	aminocaproic acid, citric acid, cycol(arginine–proline), glutamic acid dipeptide, phenylalanine, 6-glu-coclaurine
Nucleosides	cyclic adenosine monophosphate, cyclic guanosine monophosphate, guanosine hydrate, uridine monophosphate
Phenolic Acids	hydroxybenzoic acid, linoleic acid, protocatechuic acid, oleic acid
Volatile Oils	9,12-octadecadienoic acid, 9-octadecenoic acid, arachidic acid, docosanoic acid, lauric acid, myristic acid, palmitic acid, palmitoleic acid, stearic acid
Others	ebelin lactone

Table 2. Chemical constituents in Chuanxiong (Chuanxiong Rhizoma, *Ligusticum chuanxiong Hort.*).

Class	Compound
Volatile Oils	bornyl acetate, caryophyllene, germacrene D, germacrene d-4-ol, methyl-eugenol, p-cymen-8-ol, sabinene, terpinen-4-ol, tetradecanoic acid, *α*-cadinol, *α*-copaene, *α*-farnesene, *α*-phellandrene, *α*-pinene, *α*-selinene, *α*-terpinen, *α*-terpinene, *α*-terpineol, *α*-terpinolene, *α*-terprinene, *α*-thujene, *β*-chamigrene, *β*-elemene, *β*-farnesene, *β*-germacrene, *β*-linalool, *β*-myrcene, *β*-ocimenen, *β*-phellandrene, *β*-pinene, *β*-selinene, *γ*-cadinene, *γ*-gurjunene, *γ*-muurolene, *γ*-selinene, *γ*-terpinene, θ-cadinene, τ-muurolol, τ-terpinen, (+)-*β*-linalool, (1s,5s)-(-)-2(10)-pinene, 2-methoxy-4-vinylphenol, butanal, cubebene, dodecanoic acid, methyl ester hexadecanoic, n-hexadecanoic acid, n-hexadecanoic acid methyl ester, octadecanoic acid, pentadecanoic acid, pentadecanoic acid ethyl ester, spathulenol, cis,cis-linoleic acid, (E)-2-hexenal, (E)-3-decen-2-ol, (N)-(-)-p-menth-1-en-4-ol, 1n-a-pinene, limonene, n-tridecane, o-cymene, n-hexacosane, p-eugenol , p-limonene, p-menth-1-en-8-ol, p-mentha-1,4(8)-diene, p-mentha-1,4-dien-7-ol, l-(+)-ascorbic acid 2,6-dihexadecanoate, n-eicosane, carotol, cedrol, cis-3-hexen-1-ol, cis-*β*-terpineol, (-)-calamenene, (-)-spathulenol, epiglobulol, igusticoside A, igusticoside b, linoleic acid ethyl ester, linoleic acid methyl ester, (E)-2-methoxy-4-(3-(methylsulfonyl)prop-1-en-1-yl)phenol, (S)-3-ethyl-4-methylpentanol, [(Z)-1,2-diphenyleth-1enyl]-trimethylsilan, 1,1-dimthyl-1,2,3,5,7,8,9,9a-octahydro-benzocyclohepten-6-one, 1,2,3,4,4a,7-hexahydro-1,6-dimethyl-4-(1,-methylethyl)-naphthalene, 1,3,5-dodecatriene, 1,3-cyclohexadiene-5-pentyl, 1,4-cyclohexadiene-1,2-dicarboxylic anhydride, 1,5,5-trimethyl-6-methylene-cyclohexene, 1,5-copaenol, 1,7,7-trimethyl-bicyclo[2.2.1]hept-2-yl, 1-ethenyl-1-methyl-2,4-bis(1-methylethenyl)-cyclohexane, 1-hexadecanol, 1-lsopropyl-4-methylbicyclo[3.1.0]hex-2-ene, 1-methoxy adamantane, 1-methyl-4-(1-methylethenyl)-benzene, 1-methyl-4-(methylethyl)-(E)-2-cyclohexenol, 1-phenyl-1-pentanone, 2,4-decadienal, 2,5,5,8a-tetramethyl-3,5,6,7,8,8a-hexahydro-1(2h)-naphthalenone, 2,5-dimethyl-3-hexyne-2,5-diol, 2,6,10-trimethyltetradecane, 2-chloro-1-(2,4-dimethylphenyl)-2-methyl-1-propanone, 2-lsopropenyl-4a,8-dimethyl-1,2,3,4,4a,5,6,7,-octahydronaphthalene, 2-octyl-phenol, 2-pentadecanone-6,10,14-trimethyl , 3,7-dihydro-7-(2-hydroxyethyl)-1,3-dimethyl-1h-purine-2,6-dione, 3-ethyl-4-methylpentan-1-ol, 3-hydroxy-4-methoxypropiophenone, 4(10)-thujene, 4-[*β*-d-apiofuranosyl-(1→6)-*β*-d-glucopyranosyloxy]-3-methoxypropio-phenone, 4-hydroxy-2-methylacetophenone, 4-isopropylidene-1-winyl-o-menth-8-ene, 5-pentylcyclohexa-1,3-diene, 6-butyl-1,4-cycloheptadiene, 8-propoxycedrane, 9,12-octadecadienal, a-caryophyllene, angelicide, ansaspirolide, cis,trans-4-methyl-3-oxabicyclo[4.4.0]decane, cis-1-methyl-4-(1-methylethyl)-2-cyclohexen-1-ol, cis-3-buty-4-vinyl-cyclopentene, copaene, cubebol, cubenene, di-epi-1,10-cubenol, diepi-*α*-cedrene epoxide, diethyl phthalate, dodecamenthylcyclohexasiloxane, dodecanal, ethyl hexadecanoate, ethyl linoleate, heneicosane, humulane-1,6-dien-3-ol, llavandulyl propionate, methyl benzoylformate, neophytadiene, nonadecanol, pentadecyl alcohol, phthalic anhydride, phytol, squalene, tetradecanmethyl-cycloheptasiloxane, trans-1-methyl-4-(1-methylethyl)-2-cyclohexen-1-ol, trans-p-menth-1-en-3-ol, trans-sedanolide, trans-*α*-bergamotene, tropone, valerophenone, viridiflorol, 1-hydroxy-1-(3-methoxy-4-hydroxyphenyl)-ethane, 3-methoxy-4-hydroxystyrene, coniferyl ferulate, sedanonic acid, 5-hydroxymethyl-6-endo-3-ethoxy-4-hydroxyphenyl-8-oxa-bicyclo[3.2.1]-oct-3-one, succinic acid, ligubenzocycloheptanone A, (S)-2-(2-carboxyl-2-hydro-xyethylthio)-ferulic acid, caffeic acid, ferulic acid, butylidenephthalide, dicaffeoylquinic acid
Alkaloids	1-acetyl-*β*-carboline, 1-*β*-ethylacrylate-7-aldehydo-*β*-carboline, 2,3-diphenyl-1,3,4-oxadiazol-3-ium-5-olaten-pentyl-4,5-dihydrophthalide, adenine, adenosine, choline, cyclotetradecane, ligustrazine, l-isobutyl-l-valine anhydride, l-valine-l-valine anhydride, pelolyrine, tetramethylpyrazine, trimethylamine, uracil

Continued

Ceramides and Cerebrosides	(2N)-2-hydroxy-n-[(2s,3s,4n,8e)-1,3,4-trihydroxypentadec-8-en-2-yl]heptacosanamide, (2N)-2-hydroxy-n-[(2s,3s,4s,8e)-1,3,4-trihydroxyicos-8-en-2-yl]tetracosanamide, (2N)-2-hydroxy-n-(3s,4s,5s)-4-hydroxy-5-[(4e)-undec-4-en-1-yl]tetrahydrofuran-3-yl heptacosanamide, (2N)-n-[(2n,3s,4n,8e)-1-(*β*-d-glucopyranosyloxy)-3,4-dihydroxyoctadec-8-en-2-yl]-2-hydroxyhexadecanamide, (2N)-n-[(2s,3n,4e,8e)-1-(*β*-d-glucopyranosyloxy)-3-hydroxydodeca-4,8-dien-2-yl]-2-hydroxydocosanamide
Coumarins	5,7,8-trimethyl-dihydrocoumarin, scopoletin, cnidioside A
Flavonoids	apigenin, apigenin-7-o-*β*-d-glueuronide, daidzein, astragalin, 2-(1-oxopentyl)-benzoic acid methyl ester
Phenolic Acids	icariside F_2, ligusticumacid A, ligusticumacid B, ligusticumacid C, ligusticumacid d, ligusticumacid e, ligusticumacid F, 5-hydroxymethyl-6-endo-3-methoxy-4-hydroxyphenyl-8-oxa-bicyclo (3.2.1)-oct-3-one, chrysophanol, folic acid, gallic acid, palmitinic acid, p-hydroxybenzoic acid, sinapic acid, vanillin, chlorogenic acid, vanillic acid, linoleic acid, protocatechuic acid, oleic acid
Phthalides	levistilide A, ligustilide, 3-butylene-4,5-dihydroxyph-thailde, senkyunolide Q, senkyunolide R, senkyunolide S, e-senkyunolide E, ligusticoside A, z-ligustilide , z-senkyunolide E, (z,z')-6,8',7,3'-diligustilide, 3-butylidene-6-hydroxy-5,6-dihydrophthalide, 3-carboxyethyl-phthalide, 4,5-dihydro-3-butylidenephthalide, 3,6,8,3a-diligustilide, 3,8-dihydrodiligustilide, 3-butylidenephthalide, 3-hydroxy-4,5,6,7-tetrahydro-6,7-dihydroxy-3-butylphthalide, 4,5-dihydro-3-butylphthalide, 4,7-dihydroxy-3-butylphthalide, 4-hydroxy-3-butylphthalide, butylphthalide, chuanxiongins A, chuanxiongins B, chuanxiongins C, chuanxiongins d, chuanxiongins E, chuanxiongins F, chuanxiongnolide A, chuanxiongnolide B, chuanxiongnolide R_1, chuanxiongnolide R_2, hexahydro-3-butylphthalide, levistolide A, neocnidilide, senkyunolide B, senkyunolide C, senkyunolide E, senkyunolide G, senkyunolide K, senkyunolide l, senkyunolide M, senkyunolide N, senkyunolide O, senkyunolide P, tokinolide B, wallichilide, z,z'-3,3',8,8'-diligustilide, z'-3,8-dihydro-6,6',7,3'a-diligustilide, z-6,7-epoxyligustilide, z-butylidenephthalide, z-ligustilide dimer e-232, 3-butylidene-7-hydroxyphthalide, 3-carboxyrthyl-phthalide, 6-hydroxy-senkyunolide B, celephthalide A, chuanxiongoside A, chuanxiongoside B, (3'z)-(3s,8n,3a's,6'n)-4,5-dehydro-3.3a',8.6'-diligustilide, (3'z)-(3s,8s,3a's,6'n)-4,5-dehydro-3.3a',8.6'-diligustilide, thiosenkyunolide C, trans-neocridilide, chuanxiongdiolide A, chuanxiongdiolide B, chuanxiongdiolide L_1, chuanxiongdiolide L_2, chuanxiongdiolide L_3, chuanxiongdiolide L_4, chuanxiongdiolide L_5, chuanxiongdiolide R_1, chuanxiongdiolide R_2, chuanxiongdiolide R_3, igustilide, 3-butyl-4-hydroxyphthalide, 3-butylphthalide, e-ligustilide, riligustilide, senkyunolide A, senkyunolide D, senkyunolide F, senkyunolide h, senkyunolide I, senkyunolide J, z,z'-6,8',7,3'-diligustilide, cnidilide
Polysaccharides	LCA, LCB, LCC, LCX0, LCX1, LCX2, LCP-1, LCP-2, LCP-3, LCP-4, Arabinose, galactose, galacturonic acid, glucose, glucuronic acid, nhamnose, mannose
Triterpenoids	progesterone, xiongterpene, Globulol, ergosterol peroxide
Others	(-)-alloaromadendrane-4*β*,10*α*,13,15-tetrol, 3-o-*β*-d-apiofuranosyl-(1→6)-*β*-d-glucopyranoside, 4-pentylcyclohex-3-ene-1*α*,2*β*-diol, campest-4-en-3-one, monopalmitin, *β*-d-apiofuranosyl-(1→6)-*β*-d-glucopyranosyl-3,4-dimethoxy-benzoate, aurantiamide acetate, lignoceric acid

contained 18 steroids, 6 flavonoids, 4 phenylpropanoids, 2 alkaloids, and 2 benzophenones using UPLC-Q-TOF/MS combination with characteristic fragments filter and neutral loss filter method [57]. The study on chemical constitutions of crude Anemarrhenae rhizoma (CAR) and salt-processed Anemarrhenae rhizoma (SAR) showed that a total of 24 components as main contributors had significant difference, and 7 main constituents were simultaneously determined by

ultra-high-performance liquid chromatography-quadrupole mass spectrometry (UHPLC-MS), timosaponin N, timosaponin E_1, timosaponin BII, timosaponin BIII, anemarrhenasaponin I, timosaponin AII and timosaponin AIII [58].

We sorted out 163 components of Anemarrhenae rhizoma [57]-[76], which were classified into 10 categories, including steroids (84 compounds, 51%), flavonoids (25 compounds, 15%), alkaloids (13 compounds, 8%), coumarins (8 compounds, 5%), volatile oils (8 compounds, 5%), triterpenoids (5 compounds, 3%), polysaccharides (6 compounds, 4%), lignans (7 compounds, 4%), phenolic acids (1 compound, 1%) and others (6 compounds, 4%) (**Figure 1(c)**, **Table 3**).

2.4. Fuling (Poria, Poria Cocos (Schw.) Wolf.)

We used key words "Fuling", "Poria", "*Poria cocos* (*Schw.*) *Wolf.*", "*Wolfiporia extensa* (*Peck*) *Ginns*" to find the ingredients in Poria, and selected literatures in the past five years. We found many methods have been developed for analysis and quality control of Poria. For instance, qualitative and quantitative methods to analyze carbohydrates (polysaccharides, oligosaccharides and monosaccharides) in three different parts (epidermis, middle and inner) of Poria by high performance gel permeation chromatography coupled with charged aerosol detector (HPGPC-CAD), altra-performance liquid chromatography coupled with triple quadrupole mass spectrometry (UHPLC-QqQ-MS/MS), PCA and orthogonal partial least squared discriminant analysis (OPLS-DA) [77]. It is an efficient dereplication strategy to identify triterpene acid analogues in Poria based on ultra-performance liquid chromatography with electrospray ionisation quadrupole time-of-flight tandem mass spectrometry (UPLC-ESI-QTOF-MS/MS), and 62 triterpene acids were characterized [78]. A method based on UHPLC-MS combined metabolomics approach is established to explain the distribution of triterpene compounds in four parts, which are Poriae Cutis (PC), Rubra Poria (RP), White Poria (WP) and Poria cum Radix Pini (PRP) and 51 triterpene compounds are tentatively identified in Poria. The PC and PRP show a quite clear discrimination by the PCA and OPLS-DA, and 12 differential compounds are found [79].

The four classes of chemical components in Poria included 204 compounds [77]-[96], containing triterpenoids (137 compounds, 67%), polysaccharides (58 compounds, 28%), alkaloids (2 compounds, 1%) and others (7 compounds, 4%) (**Table 4**, **Figure 1(d)**).

2.5. Gancao (Licorice, *Glycyrrhiza uralensis Fisch.*, *Glycyrrhiza inflata Bat.* or *Glycyrrhiza Glabra L.*)

We used key words "Gancao", "Zhigancao", "Licorice", "*Glycorrhizae Radix et Rizoma*", "*Glycorrhiza urensis Fisch.*", "*Glycorrhiza inflata Bat.*", "*Glycorrhiza glabra L.*" to find the ingredients in Licorice, and we were mainly based on the literature of the past five years and delete the literature with duplicate components.

Table 3. Chemical constituents in Zhimu (Anemarrhenae rhizoma, *Anemarrhena asphodeloides Bge*.).

Class	Compound
Steroids	(25R)-26-o-β-d-glucopyranosyl-5α-furostane-20(22)-en-3β,26-diol-3-o-β-d-glucopyranosyl-(1→2)-β-d-glucopyranosyl-(1→4)-β-d-galactopyranoside, (25S)-26-o-β-d-glucopyranosyl-22-hydroxy-5β-furostane-3β,26-diol, 3-o-β-d-glucopyranosyl-(1→2)-o-β-d-galactopyranoside, (25S)-officinalisnin-I, 20(22)-en-5β-furost-3β,15α-diol-3-o-β-d-glucopyranosyl-(1→2)-β-d-galactopyranoside, 21-formyl sarsasapogenin, 21-hydroxysarsasapogenin, 25(27)-ene-anemarrhenasaponin I, 25(27)-ene-Gurilioside H, 25(27)-ene-timosaponin AII, 25(27)-ene-timosaponin AIII, 25(27)-ene-timosaponin N, 25R-timosaponin AIII, 25R-timosaponin BII, 25R-timosaponin BIII, 25R-timosaponin D, 25R-timosaponin E, 25S-karatavioside C, 2-hydroxyl-timosaponin AIII, 3,4-dihydroxyallylbenzene-3-o-α-L-rhamnopyranosyl(1→6)-β-d-glucopyranoside, anemarnoside A, anemarnoside B, anemarrhena S_1, anemarrhena S_2, anemarrhena S_3, anemarrhena S_4, anemarrhena S_5, anemarrhena saponin III, anemarrhenasaponin F, anemarrhenasaponin I, anemarrhenasaponin Ia, anemarrhenasaponin II, anemarsaponin B II, asparagoside G, curilioside H, degalactotigonin, desgalactotigonin, dimethisterone, diuranthoside A, f-gitonin, filicinoside-A, hostaplantagineoside C, karatavioside C, macrostemonoside F, macrostemonoside J, officinalisinin-I, petunioside N, purpureagitoside, sarsasapogenin, smilageninoside, solanigroside F, spicatoside B, timopregnane A, timosaponin AI, timosaponin AII, timosaponin AIII, timosaponin AIV, timosaponin B, timosaponin B I, timosaponin B II, timosaponin B III, timosaponin B IV, timosaponin C, timosaponin D, timosaponin E, timosaponin E_1, timosaponin F, timosaponin G, timosaponin H_1, timosaponin I_1, timosaponin I_2, timosaponin L, timosaponin N, timosaponin P, timosaponin Q, timosaponin U, timosaponin V, timosaponin W, timosaponin E_1, tomatoside A, tuberoside G, uttroside B, xilingsaponin B, zimoside A
Alkaloids	adenosine, coumaroyl tyramine, cyclo dileucyl, cyclo hetaleucyl, cyclo hexaleucyl, cyclo nonaleucyl, cyclo octaleucyl, cyclo pentaleucyl, cyclo tetraleucyl, cyclo trileucyl, n-cis-feruloyl tyramine, n-trans-feruloyltyramine, tryptophan
Polysaccharides	d-galactose, d-galacturonic acid, d-glucose, d-mannose, l-arabinose, l-rhamnose
Phenolic Acids	oleic acid
Flavonois	(2,6-dihydroxy-3-(((1s,4s,6r)-4-hydroxy3,7-dioxabicyclo[4.1.0]heptan-4-yl)methyl)-4-methoxyphenyl)(4-hydroxyphenyl)methanone, (2s)-7,4′-dihydroxy-5-methoxyflavone, (4-hydroxyphenyl)(2,3,4-trihydroxyphenyl)methanone, (s)-5-(2,4-dihydroxy-3-(4-hydroxybenzoyl)-6-methoxyphenyl)pyrrolidin-2-one, 2,4',6-trihydroxy-4-methoxy benzophenone, 2,6,4'-trihydroxy-4-methoxy Benzophenone, 2',4',4-trihydroxy chalcone, 2'-o-methylisoliquiritigenin, 2'-o-methylphloretin, 4',6-dihydroxy-4-methoxybenzophenone-2-o-(2''),3-c-(1'')-1''-desoxy-α-l-fructofuranoside, anemarchalconyn, e-4'-demethyl-6-methyleucomin, E-5,7-dihydroxy-3-(4'-hydroxybenzylidene)chroman-4-one, foliamangiferoside A, iriflophene, iriflophenone, iriflpohenone-3-c-β-d-glucoside, isomangiferin, isosakuranetin, isovitexin, mangiferin, mangiferoxanthone A, methyl 2-(2,4-dihydroxy-3-(4-hydroxybenzoyl)-6-methoxyphenyl) acetate, neomangiferin, vitexin
Triterpenoids	platycodin D, platycodin D_2, platycodin D_3, platycoside A, polygalacin D_2
Volatile Oils	dihydroxy-octadecadienoic acid, dihydroxy-octadecaenoic acid, dihydroxy-octadecatrienoic acid, hydroxy-octadecadienoic acid, hydroxy-octadecatrienoic acid, inolenic acid, tearic acid, trihydroxy-octadecaenoic acid
Coumarins	cis-hinokiresinol, hinokiresinol, methoxy-cis-hinokiresinol, monomethyl-cis-hinokiresinol, oxy-hinokiresinol, trans-hinokiresinol, broussonin A, broussonin B
Lignans	3''-hydroxy-4''-methoxy-4''-dehydroxynyasol, 3''-methoxynyasol, 4-hydroxy acetophenone, 4-hydroxybenzaldehyde, 4'-o-methylnyasol, anemarcoumarin A, nyasol,
Others	(s)-5-(2-hydroxy-5-(3-(4-hydroxy phenyl) propyl)-4-methoxy phenyl) pyrrolidin-2-one, aneglycoside A, aneglycoside B, aneglycoside C, phytosphingosine, α-d-Glucose monoallyl ether

Table 4. Chemical constituents in Fuling (Poria, *Poria cocos* (*Schw.*) *Wolf*).

Class	Compound
Polysaccharides	ab-PCM3-I-S1, ab-PCM3-I-S2, ab-PCM3-I-S3, ab-PCM3-I-S4, ab-PCM3-I-S5, ac-PCM2, ac-PCM3-I-S1, ac-PCM3-I-S2, ac-PCM3-I-S3, ac-PCM3-I-S4, ac-PCM3-I-S5, arabinose, carboxymethylated P. cocos polysaccharides, carboxymethylpachymaram, CMP, CS-PCS3-II, fucose, galactose, galacturonic acid, glucose, glucuronic acid, mannose, oxidized P. cocos polysaccharides, pachyman, PC-II, PCP3-I, PCP3-II, PCP4-I, PCP4-II, PCP-E, PCP-H, PCP-M, PCPS, PCP-U, PCS1, PCS2, PCS3-I, PCS3-II, PCS4-I, PCS4-II, PCSC, PCWPS, PCWPW, Pi-PCM, Pi-PCM0, Pi-PCM1, Pi-PCM2, Pi-PCM3-I, poria cocos polysaccharide-1(PCP-1), PPSW-1, rhamnose, rib, ribose, sum, wc-PCM0, wc-PCM1, wc-PCM2, xylose
Triterpenoids	11*β*-ethoxydaedaleanic acid A, 15*α*-hydroxy-3-oxolanosta-8,24-dien-21-oic acid, 15*α*-hydroxydehydrotrametenolic acid, 15*α*-hydroxydehydrotumulosic acid, 15*α*-hydroxyeburiconic acid, 16-deoxyporicoic acid B, 16-hydroxy-3,24-dioxolanosta-7,9-dien-21-oic acid, 16-o-acetyl pachymic acid, 16*α*,25-dihydroxy dehydroeburiconic acid, 16*α*,25-dihydroxyeburiconic acid, 16*α*,27-dihydroxydehydrotrametenoic acid, 16*α*,29-dihydroxy eburiconic acid, 16*α*-acetoxy-26,27-dimethoxyl-lanosta-8,24-dien-21-oic acid, 16*α*-acetoxy-lanosta-7,9,24-dien-21-oic acid, 16*α*-acetoxy-lanosta-8,24-dien-21-oic acid, 16*α*-acetoxypolyporenic acid C, 16*α*-acetyloxy-24-methylene-3-oxolanosta-7,9-dien-21-oic acid, 16*α*-acetyloxye buriconic acid, 16*α*-hydroxy-3-oxolanosta-8,24-dien-21-oic acid, 16*α*-hydroxydehydrotrametenonic aicd, 16*α*-hydroxy eburiconic acid, 16*α*-hydroxy-lanosta-7,9,24-trien-21-oic acid, 16*α*-hydroxy-lanosta-8,24-dien-21-oic acid, 16*α*-hydroxy trametenolic acid, 16,29-dihydroxy-3,4-seco-lanosta-4(28),8,24(31)-trien-3,21-dioic acid, 2'-3'-dihydrosorbicillin, 25,26-dihydroxy dehydropachymic acid, 25-hydroxy-3-epidehydrotumulosic acid, 25-hydroxy-3-epitumulosic acid, 25-hydroxy pachymic acid, 25-hydroxy polyporenic acid C, 25-hydroxyporicoic acid C, 25-hydroxyporicoic acid H, 25-methoxy-29-hydroxy poricoic acid HM, 25-methoxy poricoic acid A, 25*α*-hydroxytumulosic acid, 26-hydroxyporicoic acid DM, 26-hydroxy poricoic acid G, 29-hydroxydehydropachymic acid, 29-hydroxydehydrotumulosic acid, 3-(2-hydroxy acetoxy)-5*α*,8*α*-peroxydehydrotumulosic acid, 3,4-seco-lanosta-4,7,9,24-tetraen-3,21-dioic acid, 31-hydyoxyl-16-o-acetylpachymic acid, 3-acetyloxy-16*α*-hydroxy trametenolic acid, 3-epi-(3'-hydroxy-3'-methylglutaryloxyl)-dehydrotumulosic acid, 3-epi-dehydropachymic acid, 3-epi-dehydrotumulosic acid, 3-epi-pachymic acid, 3-o-acetyl-16*α*,26-dihydroxytrametenolic acid, 3-o-acetyl-16*α*-hydroxydehydrotrametenolic acid, 3-o-acetyl-16*α*-hydroxytrametenolic acid, 3-o-acetyl-dehydroeburicoic acid, 3-oxo-16*α*-hydroxy-lanosta-7,9(11),24-trien-21-oic acid, 3-oxo-6,16*α*-dihydroxy-lanosta-7,9(11),24(31)-trien-21-oic acid, 3-oxo-lanosta-7,9(11),24(31)-trien-21-oic acid, 3-oxo-lanosta-7,9(11),24-trien-21-oic acid, 3*α*,16*α*,25-Trihydroxylanosta-8,24-dien-21-oic acid, 3*β*,15*α*-Bis(acetyloxy)-24-methylenelanost-8-en-21-oic acid, 3*β*,15*α*-dihydroxy-24-oxolanosta-8-en-21-oic acid, 3*β*,15*α*-dihydroxy lanosta-7,9(11),24-triene-21-oic acid, 3*β*,16*α*-bis(acetyloxy)-29-hydroxylanosta-8,24-dien-21-oic acid, 3*β*,16*α*-dihydroxy-24-hydroxy methyl lanosta-7,9-dien-21-oic acid, 3*β*,16*α*-dihydroxy lanosta-7,9(11),24(31)-trien-21-oic acid, 3*β*,16*α*-dihydroxylanosta-7,9(11),24-trien-21-oic acid, 3*β*,26-dihydroxy-lanosta-7,9,24-trien-21-oic acid, 3*β*-acetoxy-16*α*,26-dihydroxylanosta-8,24-dien-21-oic acid, 3*β*-acetoxy-lanosta-7,9(11),24-trien-21-oic acid, 3*β*-acetyloxy-16*α*-hydroxy-24-oxolanost-7,9(11)-dien-21-oic acid, 3*β*-acetyloxy-16*α*-hydroxy-24-oxolanost-8-en-21-oic acid, 3*β*-hydroxy-16*α*-acetoxylanosta-7,9(11),24-trien-21-oic acid, 3*β*-p-hydroxybenzoyldehydrotumulosic acid, 3*α*-hydroxyl-26-methoxyl-8,24(31)-diene-21-oic acid, 3*β*-acetoxy-16*α*-hydroxy-lanosta-8,24-diene-21-oic acid, 5*α*,8*α*-Peroxydehydrotumulosic acid, 6,16*α*-dihydroxydehydroeburiconic acid, 6,16*α*-dihydroxydehydrotrametenonic acid, 6-hydroxy polyporenic acid C, 6*α*-hydroxydehydropachymic acid, 6*α*-hydroxy-polyporenic acid C, 9,11-dehydroergosterol peroxide, 6-hydroxy-3*β*,16*α*-diacetoxy-lanosta-7,9,24-tetraen-21-oic acid, acetoxyeburicoic acid, acetyl-eburicoic acid, ceanphytamic acid A, ceanphytamic acid B, daedaleanic acid A, daedaleanic acid B, dehydroeburic acid, dehydroeburicoic acid, dehydroeburicoic acid monoacetate, dehydroeburiconic acid, dehydropachymic acid, dehydrosulphurenic acid, dehydrotrametenolic acid, dehydrotrametenonic acid, dehydrotumulosic acid, dehypachymic acid, deyhdrotarmetenolic acid, eburicoic acid, eburicoic acid acetate, ergosterol

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	peroxide, harzianone, hederagenin, hispindic acid B, lanosta-7,9,24-trien-21-oic acid, lanosta-8,24-dien-21-oic acid, me trametenolate, oleanic acid, pachymic acid, pinicolic acid, pinicolic acid A, pinicolic acid E, polyporenic acid C, poriacosone A, poriacosone B, poricoic acid A, poricoic acid AE, poricoic acid AM, poricoic acid B, poricoic acid C, poricoic acid CE, poricoic acid CM, poricoic acid D, poricoic acid DM, poricoic acid E, poricoic acid F, poricoic acid G, poricoic acid GE, poricoic acid GM, poricoic acid h, poricoic acid HE, poricoic acid HM, poricoic B, pregna-7-en-3α,11α,15α,20-quadriol, trametenolic acid, tumulosic acid, ursolic acid
Alkaloids	adenosine, thymine
Others	(3S,6S)-3-[(1R)-1-hydroxyethyl]-6-(phenylmethyl)-2,5-piperazinedione, (5-formylfuran-2-yl) methyl 2-(4-hydroxyphenyl) acetate, (5-formylfuran-2-yl) methyl 2-hydroxy propanoate, 5-hydroxy methylfurfural, sohiracillinone, sohirnone A, sorbicillin

Many methods have been developed for the analysis and the quality control of Licorice. The 58 phenolic acids including 11 new compounds (glycybridins A-K) were isolated from Licorice using nuclear magnetic resonance (NMR) and MS analyses combination with experimental and computed electronic circular dichroism data [97]. The 8 triterpenoids including glyuralsaponin A-G were separated from Licorice root by using ultraviolet (UV), infrared (IR), NMR spectrum and other technologies [98]. In addition, 122 chemical ingredients including glycyuralin A-F from the roots and stems of Licorice were isolated and identified adopting a combined strategy using NMR and MS spectroscopic data measurement [99].

We totally collected 260 components of Licorice [97]-[109], which were classified into 6 categories, including flavonoids (112 compounds, 43%), triterpenoids (51 compounds, 20%), phenolic acids (72 compounds, 28%), coumarins (11 compounds, 4%), stilbenoids (5 compounds, 2%) and others (9 compounds, 3%) (**Figure 1(e)**, **Table 5**).

3. The Chemical Constituents in Suanzaoren Decoction

We used "Suanzaoren decoction", "Suan-Zao-Ren decoction", "Suan-Zao-Ren-Tang", "Zizyphus Combination", "Suanzaoren Formulae", "Suan Zao Ren Tang", "Ziziphus spinose decoction", "Suanzaoren prescription" as key words to search chemical components in SZRD, and classified them according to their chemical structure. Results showed that only five literatures on the ingredients of SZRD were reported. For example, 101 constituents are identified in Suan-Zao-Ren granule using UHPLC-Q-TOF-MS coupled with multiple data processing approaches [110]. These 48 components were characterized in SZRD using liquid chromatography time-of-flight mass spectrometry (LC-Q/TOF-MS) and liquid chromatography coupled with ion trap mass spectrometry (LC-IT-MS) technology and 31 compounds were identified for the first time [111]. The 22 chemical compounds were isolated in SZRD by using ultra-performance liquid chromatography coupled with electrospray ionization/quadrupole-time-of-flight mass spectrometry (UPLC-ESI-Q-TOF-MS)

Table 5. Chemical constituents in Gancao (Licorice, *Glycyrrhiza uralensis Fisch.*, *Glycyrrhiza inflata Bat. or Glycyrrhiza glabra L.*).

Class	Compound
Flavonoids	7,4'-dihydroxyflavone 7-O-glucopyranoside, apigenin 6-C-rhamnopyranoside-8-C-[6'''-(3-hydroxy-3-methylglutaroyl)]-glucopyranoside, daidzin, glucoliguiritin apioside, glycyroside, isoliquiritigenin 4,4'-di-O-glucopyranoside, isoliquiritin, isoschaftoside, isoviolanthin, liquiritigenin 7,4'-di-O-glucopyranoside, liquiritin, liquiritin apioside, neoisoliquiritin, neoliquiritin, schaftoside, soliquiritin apioside, sophoraflavone B, syringic acid 4-O-glucopyranoside, vicenin II, (2R,3R)-3,4',7-trihydroxy-3'-prenylflavane, (2s)-abyssinone I, 2,3-dehydrokievitone, 3,4-didehydroglabridin, 3'-hydroxy-4'-O-methylglabridin, 3-hydroxyglabrol, 4'-O-methylglabridin, 5,8-dihydroxy-flavone-7-O-beta-D-glucuronide, 5-hydroxy-8-methoxyl-flavone-7-O-beta-D-glucuronide, 6-prenylnaringenin, 7,2'-dihydroxy-4'-methoxy-8-(3-methyl-2-butenyl) isoflavanone, 8-prenyl-phaseollinisoflavan, abyssinone II, chalcones isoliquiritin, derrone, echinatin, euchrenone A_5, flavanone, flavonol, formononetin, galbrene, genistein, glabranin, glabrene, glabridin, glabroisoflavanone A, glabroisoflavanone B, glabrol, glabrone, glisoflavanone, glucoliquiritin apioside, glychionide A, glychionide B, glycyrrhisoflflavone, glyinflanin H, glyzarin, hispaglabridin A, hispaglabridin B, isobavachalcone, isoflavan, isoflavanone, isoflavone, isolicoflavonol, isoliquiritigenin, isoliquiritin apioside, isoononin, isoquercitrin, isotrifoliol, isoviolantin, kanzonol B, kanzonol C, kanzonol U, kanzonol W, kanzonol X, kanzonol Y, kanzonol Z, kumatakenin, licochalcone A, licochalcone B, licochalcone C, licochalcone D, licochalcone E, licoflavanone, licoflavonol, licoflflavone A, licoflflavone B, licoflflavone C, licoisoflavones A, licoisoflavones B, licoisoflflavone A, licorice glycoside A, licorice glycoside B, licorice glycoside C_1, licorice glycoside C_2, licorice glycoside D_1, licorice glycoside D_2, licuraside, liquiritigenin, lupiwighteone, mangiferin, medicarpin, naringenin, ononin, paratocarpins B, parvisoflavones A, pinocembrin, prunetin, retrochalcones, rhamnoliquiritin, shinflavanone, shinpterocarpin, violantin, wighteone
Triterpenoids	12-acetoxyganoderic acid F, 18β-glycyrrhetinic acid, 22β-acetoxylglycyrrhizin, 24-hydroxy-licorice saponin A3, 3β-O-[β-D-glucuronopyranosyl-(1→2)-β-D-glucuronopyranosyl]-24-hydroxylglabrolide, glabrolide, glycyrrhetic acid, glycyrrhetic acid-3-O-mono-β-d-glucuronide, glycyrrhizic acid, glyuralsaponin A, glyuralsaponin B, glyuralsaponin C, glyuralsaponin D, glyuralsaponin E, glyuralsaponin F, glyuralsaponin G, glyuralsaponin H, isoglycyrrhizin, licorice saponin A_3, licorice saponin B_2, licorice saponin C_2, licorice saponin E, licorice saponin E_2, licorice saponin G_2, licorice saponin N_4, squasapogenol GP-B_2, uralsaponin B, uralsaponin F, yunganoside K_2, 22β-acetoxyglycyrrhizin, 22β-acetoxyl-glycyrrhaldehyde, 3-O-glucuronopyranosyl-glycyrrhetinic acid, 3β-O-[glucuronopyranosyl-(1→2)-glucuronopyranosyl]-olean-9,12-diene-30-oic-acid, araboglycyrrhizin, glycyrrhetinic acid, glycyrrhizin, licorice-saponin H_2, licorice-saponin J_2, uralsaponin C, uralsaponin M, uralsaponin N, uralsaponin P, uralsaponin Q, uralsaponin R, uralsaponin S, uralsaponin T, uralsaponin U, uralsaponin V, uralsaponin W, uralsaponin X, uralsaponin Y
Phenolic acids	11b-hydroxy-11b,1-dihydromedicarpin, 1-methoxyphaseollin, 2'-hydroxyisolupalbigenin, 2-one-4-methoxy-pyran, 3-methoxy-9-hydroxy-pterocarpan, 5,7,4'-trihydroxy-3'(3-methylbut-2-enyl)-3-methoxy flavone, 6,8-diprenylgenistein, 6-C-prenylorobol, 7-methoxy-2',4'-dihydroxy isoflavone, 7-O-methylluteone, abiochanin A, allolicoisoflavone B, angustone A, daidzein, dehydroglyasperin C, dehydroglyasperin D, gancaonin I, gancaonin L, genkwanin, glicophenone, glicoricone, glyasperin C, glyasperin D, glycybridins A, glycybridins B, glycybridins C, glycybridins D, glycybridins E, glycybridins F, glycybridins G, glycybridins H, glycybridins I, glycybridins J, glycybridins K, glycyfuranocoumarin A, glycyfuranocoumarin B, glycyfuranocoumarin C, glycyfuranocoumarone A, glycyrol, glycyrrhiza-isoflavone C, glycyuralin A, glycyuralin B, glycyuralin C, glycyuralin D, glycyuralin E, glycyuralin F, glyurallin A, hirtellanine I, homobutein, isoangustone A, isoderrone, isoglabrone, isoglycycoumarin, isoglycyrol, isolupalbigenin, kaempferol, kaempferol 3-O-methyl ether, kumatakenin B, licoarylcoumarin, licocoumarone, licoflavone A, licoisoflavanone, licoisoflavone A, licoisoflavone B, licoricidin, licoricone, luteone, phaseollin, pratensein, semilicoisoflavone B, topazolin, uralenol

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Stilbenoids	dihydro-3,3',4'-trihydroxy-5-O-isopentenyl-6-isopentenylstilbene, dihydro-3,3'-dihydroxy-5β-d-O-glucopyranosyloxy-4'-methoxystilbene, dihydro-3,5,3'-trihydroxy-4'-methoxystilbene, dihydro-3,5-dihydroxy-4'-acetoxy-5'-isopentenylstilbene, dihydrostilbenes
Coumarins	glabrocoumarin, glabrocoumarone A, glabrocoumarone B, glycocoumarin, glycycoumarin, glycyrin, herniarin, licofuranocoumarin, licopyranocoumarin, liqcoumarin, umbelliferone
Others	[6",6"-dimethyl pyrano (2",3":4,5)]-3'-γ,γ-dimethylallyl-2',3,4'-trihydroxychalcone, 1",2"-dehydrocyclokievitone, 2'-O-demethybidwillol, dehydroglyceollin I, erybacin, licoagrocarpin, licoagrochalcone A, xambioona, gup-II

method with MassLynx™ MassFragment [112]. There were 13 active marker compounds (neomangiferin, mangiferin, spinosin, liquiritin apioside, liquiritin, 6'''-feruloylspinosin, senkyunolide I, timosaponin BII, isoliquiritoside, timosaponin C, jujuboside A, jujuboside B, and timosaponin AIII) were separated in diverse SZRD including lab-prepared Suanzaoren oral liquid, Suanzaoren mixture, and clinical Suanzaoren granules using high-performance liquid chromatography with diode array detection and evaporative light scattering detection (HPLC-DAD-ELSD) [113]. In addition, 11 chemical components (neomangiferin, mangiferin, spinosin, liquiritin apioside, liquiritin, fumalic acid, 6'''-feruloylspinosin, senkyunolide I, isoliquiritin, glycyrrhizic acid and senkyunolide A) of SZRD in different batches, including SZRD extracts, lab-made SZRD granules and clinical medicine, were determined by HPLC-PDA, which indicated that HPLC-PDA method would be helpful to improve quality evaluation and quality control in productive processes [114]. The 8 compounds (jujuboside, spinosin, ferulic acid, senkyunolide I, sarsasapogenin, mangiferin, liquiritoside and glycyrrhizic acid) were considered chemical quality control standard of SZRD [115].

We totally collected 145 compounds of SZRD in all [110] [111] [112] [113] [114], which were divided into 10 categories, including flavonoids (53 compounds, 36%), alkaloids (2 compounds, 1%), coumarins (7 compounds, 5%), phenolic acids (4 compounds, 3%), phthalide (14 compounds, 10%), triterpenoids (33 compounds, 23%), steroids (19 compounds, 13%), volatile oils (7 compounds, 5%), polysaccharide (1 compound, 1%) and others (5 compounds, 3%) (**Figure 1(f)**, **Table 6**). Flavonoids, one of the main active ingredients found at present, contained the largest number of ingredients in SZRD [116].

4. Comparative Analysis of Chemical Constituents in Herbs and Suanzaoren Decoction

4.1. Comparative Analysis of Chemical Constituents in Herbs

We obtained a total of 1104 chemical components without duplication in 5 herbs (**Figure 2(a)**). Adenosine existed in Ziziphi Spinosae Semen, Chuanxiong Rhizoma, Poria and Anemarrhenae rhizoma. Oleic acid existed in Anemarrhenae

Table 6. Chemical constituents in suanzaoren decoction.

Class	Compound
Flavonoids	(3R)-Vestitol, 5,7-Dihydroxyflavanone, 6''-Feruloyspinosin, 6''''-(4''''-O-β-D-glucopyranosyl)vanilloyl spinosin, 6,8-di-C-glucosyl-2(R)-naringenin, 6,8-di-Cglucosyl-2(s)naringenin, 6'''-Feruloylspinosin, 6''''-dihydrophaseoylspinosin, 6''-feroylspinol, choerospondin, echinatin, formononetin, gancaonin A, gancaonin H, glabrene, glabrol, glucoliquiritin, glucosylvitexin, glyinflanin A, isoliquiritigenin, isoliquiritin, isolquiritigenin, isoshaftoside, isoviolanthin, isovitexin, kanzonol F, kanzonol H, kumatakenin, licochalcone A, licochalcone B, licoleafol, licorice glycoside A, licorice glycoside D_1, licorice glycoside D_2, licorice glycoside E, licoricidin, licorisoflavan A, liquiritigenin, liquiritin, liquiritin apioside, lupiwighteone, mangiferin, neolicuroside, neomangiferin, nicotiflorin, ononin, pinocembrin, saponarin, schaftoside, shaftoside, spinosin, swertisin, vicenin II
Triterpenoids	16-Deoxyporicoic acid B, 16α-Hydroxytrametenolic acid, 22-Acetoxyglycyrrhizin, 3β,16α-Dihydroxylanosta-7,9(11),24-trien-21-oic acid, apioglycyrrhizin, betulinic acid, dehydrotrametenolic acid, dehydrotrametenonic acid, dibutyluralsaponin A, glabric acid, glycyrrhetic acid, glycyrrhizic acid, glycyrrhizin, isoliquiritoside, jujuboside A, jujuboside B, liangwanoside I, licorice saponin H_2, licorice saponin J, licorice saponin K_2, licorice saponine A_3, licorice saponine G_2, licoricesaponin B_2, oleanolic acid, pachymic acid, polyporenic acid C, poricoic acid A, poricoic acid B, uralsaponin A, uralsaponin B, yunganoside J_1, yunganoside K_2, yunganoside L_1
Coumarins	glycycoumarin, glycyrin, glycyrol, hedysarimcoumestan, inflacoumarin A, isoglycyrol, neoglycyrol
Steroids	(25s)-26-O-β-D-Glucopyranosyl-22-hydroxy-5β-furostane-3β, 26-diol3-O-β-D-Glucopyranosyl-(1-2)-O-β-D-galactopyranoside, (25s)-26-O-β-D-Glucopyranosyl-22-hydroxy-5β-furostanolalkenyl-3β, 26-diol3-O-β-D-glucopyranosyl-(1-2)-O-β-D-galactopyranoside, anemarsaponin B, anemarsaponin C, anemarsaponin E, anemarsaponin F, anemarrhena saponin II, anemarrhena saponin I, diosgenin, sarsasapogenin, timosaponin AI, timosaponin AIII, timosaponin AIV, timosaponin B II, timosaponin C, timosaponin E1, timosaponin E, timosaponin N, xilingsaponin B
Phenylpropanoids	timobiose
Phenolic Acids	chlorogenic acid, vanillic acid, neochlorogenic acid, ryptochlorogenic acid
Phthalide	3-Butyl-4-hydroxyphthalide, 3-Butylphthalide, cnidilide, E-butylidenephthalide, E-ligustilide, riligustilide, senkyunolide A, senkyunolide D, senkyunolide F, senkyunolide H, senkyunolide I, senkyunolide J, Z,Z'-6,8',7,3'-diligustilide, Z-ligustilide
Alkaloids	amphibine D, magnoflorine
Volatile oils	butylidenephthalide, caffeic acid, dicaffeoylquinic acid, ferulic acid, fumalic acid, neochlorogenic acid, palmitic acid, yptochlorogenic acid
Others	3β-30-hydroxy-11,30-dioxoolean-12-en-3-yl-2-O-a-L-arabinopyrano-syl-b-Dglucopyranosiduronic acid, emodin, liguiritigenin-7-O-β-D-apiofuransyl-40-O-3-D-glucose, ligustilide dimer, unganoside K_2

rhizoma, Chuanxiong Rhizoma and Ziziphi Spinosae Semen.

There are 8 common components presenting in Chuanxiong Rhizoma and Poria, which are galacturonic acid, mannose, arabinose, ergosterol peroxide, rhamnose, galactose, glucuronic acid, and glucose. Ziziphi Spinosae Semen and Chuanxiong Rhizoma contain linoleic acid and protocatechuic acid. Ziziphi Spinosae Semen and Anemarrhenae rhizoma contain tryptophan and isovitexin.

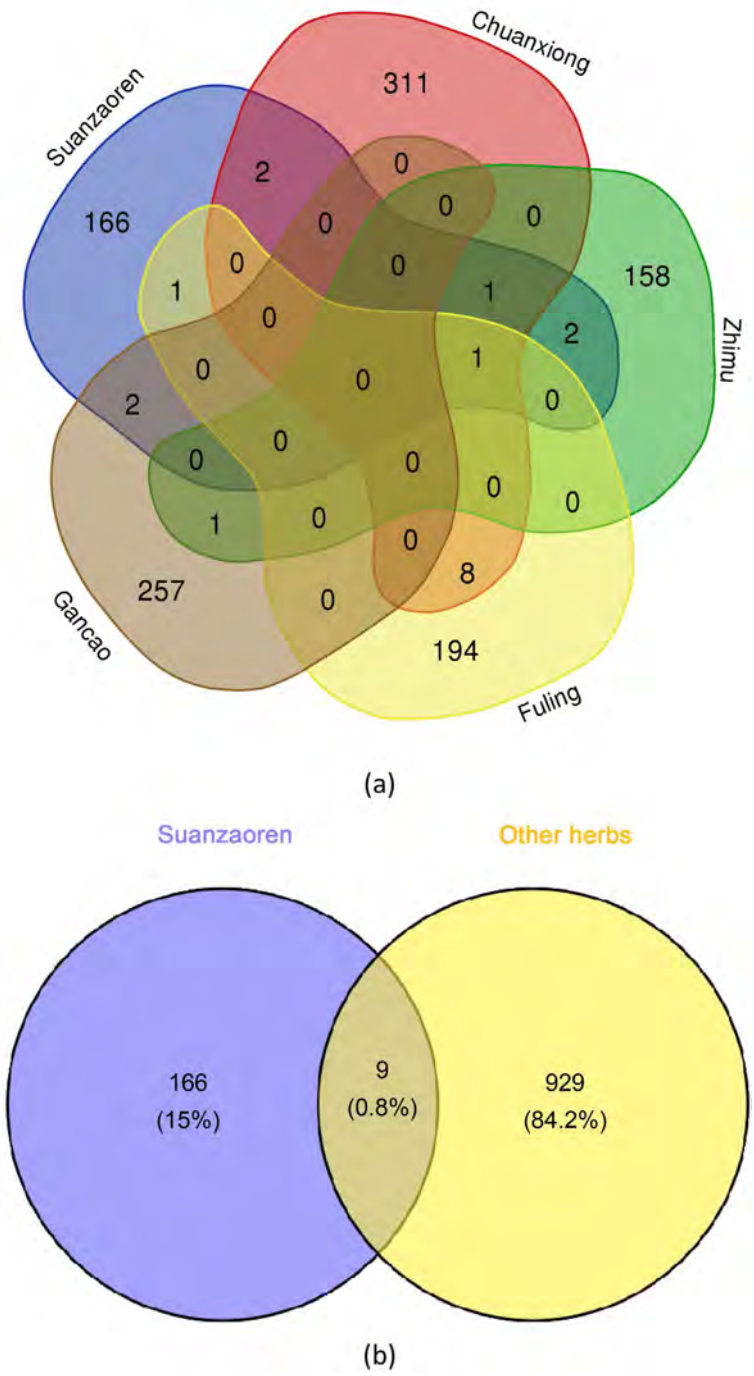

Figure 2. Chemical compounds in five-single herbs of Suanzaoren decoction. (a) Intersection of compounds in five-single herbs; (b) Intersection of compounds between Ziziphi Spinosae Semen and other four herbs.

Ziziphi Spinosae Semen and Licorice include isoquercitrin and vicenin II. Ziziphi Spinosae Semen and Poria contain ursolic acid. Anemarrhenae rhizoma and Licorice contain only one component mangiferin (**Figure 2(a)**).

In SZRD, Ziziphi Spinosae Semen seed is "Jun" (the monarch). There are 175 chemical compounds in Ziziphi Spinosae Semen seed, accounting for 15.8% in

all 5 herbs (Figure 2(b)). When compared with total compounds in the other four herbs, there are 9 common compounds with 0.8% of total compounds in all 5 herbs, including adenosine, tryptophan, isoquercitrin, isovitexin, vicenin II, linoleic acid, oleic acid, protocatechuic acid and ursolic acid (Figure 2(b)).

4.2. Comparative Analysis of Chemical Constituents between Suanzaoren Decoction and Herbs

4.2.1. Ingredients between Suanzaoren Decoction and Herbs

After comparing 145 compounds in SZRD with 1104 in 5 herbs, We found there were 80 common components which accounted for 6.8% of total compounds in all herbs and 55.2% of compounds in SZRD (Figure 3(a)), containing 7 categories, including 27 flavonoids, 17 triterpenoids, 12 steroids, 5 volatile oils, 4 coumarins, 14 phenolic acids and 1 alkaloid (Figure 3(b)) and found that 14 compounds came from Ziziphi Spinosae Semen, 18 from Chuanxiong Rhizoma, 15 from Anemarrhenae rhizoma, 9 from Poria and 27 from Licorice.

In addition, 65 compounds in SZRD are not contained in Ziziphi Spinosae Semen, Anemarrhenae rhizoma, Chuanxiong Rhizoma, Poria and Licorice (Figure 3(c)). These distinctive compounds account for 5.6% of total compounds in all herbs and 44.8% of SZRD compounds (Figure 3(a)), and include alkaloids (1 compound), coumarins (3 compounds), flavonoids (25 compounds), phenolic acids (1 compound), phthalides (2 compounds), polysaccharides (1 compound), steroids (7 compounds), triterpenoids (17 compounds), volatile oils (3 compounds) and others (5 compounds). The number of flavonoids is the highest among SZRD, followed by triterpenoids, suggesting ingredients of SZRD are not simply the sum of the single ingredients and new compounds will produce after decocting SZRD.

The amounts of volatile oils, alkaloids and polysaccharides are decreased while the number of flavonoids is increased in SZRD compared with 5 single herbs (Figure 4(a) and Figure 4(b)). In general, there are many water-soluble compounds in SZRD, such as flavonoids, triterpenoids, accounting for 59.31%. However, triterpenoids, flavonoids and volatile oils account for 22.95%, 18.24% and 15.93% respectively in 5 single herbs. Amino acids, ceramides and cerebrosides, lignans, nucleotides, steroids and stilbenides are not found in SZRD. We inferred it might due to the extraction of SZRD just exists in aqueous decoction, the proportion of components with larger polarity is significantly increased, while the proportion of components with weak polarity is relatively low. On the other hand, it may because the lack of literature on the components of SZRD, and the literatures are just for the analysis of specific components and lack researches on specific components.

4.2.2. Ingredients between Suanzaoren Decoction and Ziziphi Spinosae Semen

We compared 145 compounds contained in SZRD with 175 in Ziziphi Spinosae Semen, the monarch of SZRD (Figure 4(c)). There are 14 common components,

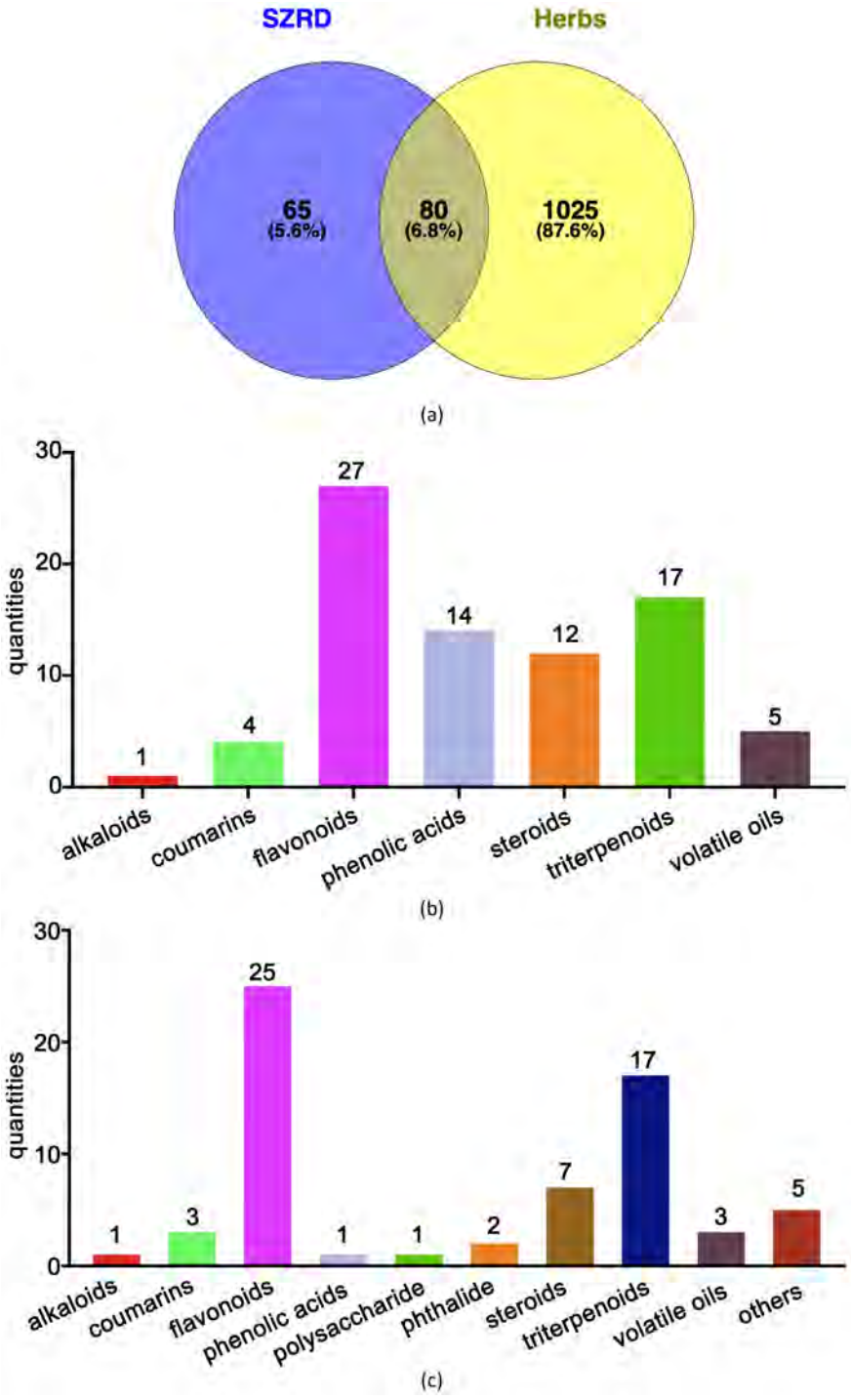

Figure 3. Chemical compounds in suanzaoren decoction and its herbs. (a) Intersection of compounds in SZRD and its herbs; (b) Classification of same compounds of SZRD and its herbs; (c) Classification of different compounds of SZRD and its herbs.

accounting for 9.7% in SZRD, which are flavonoids (6'''-Feruloylspinosin, glucosylvitexin, isovitexin, nicotiflorin, saponarin, spinosin, wertisin, vicenin II), triterpenoids (betulinic acid, jujuboside A, jujuboside B, oleanolic acid), alkaloid (magnoflorine) and volatile oil (palmitic acid), while it has 131 different

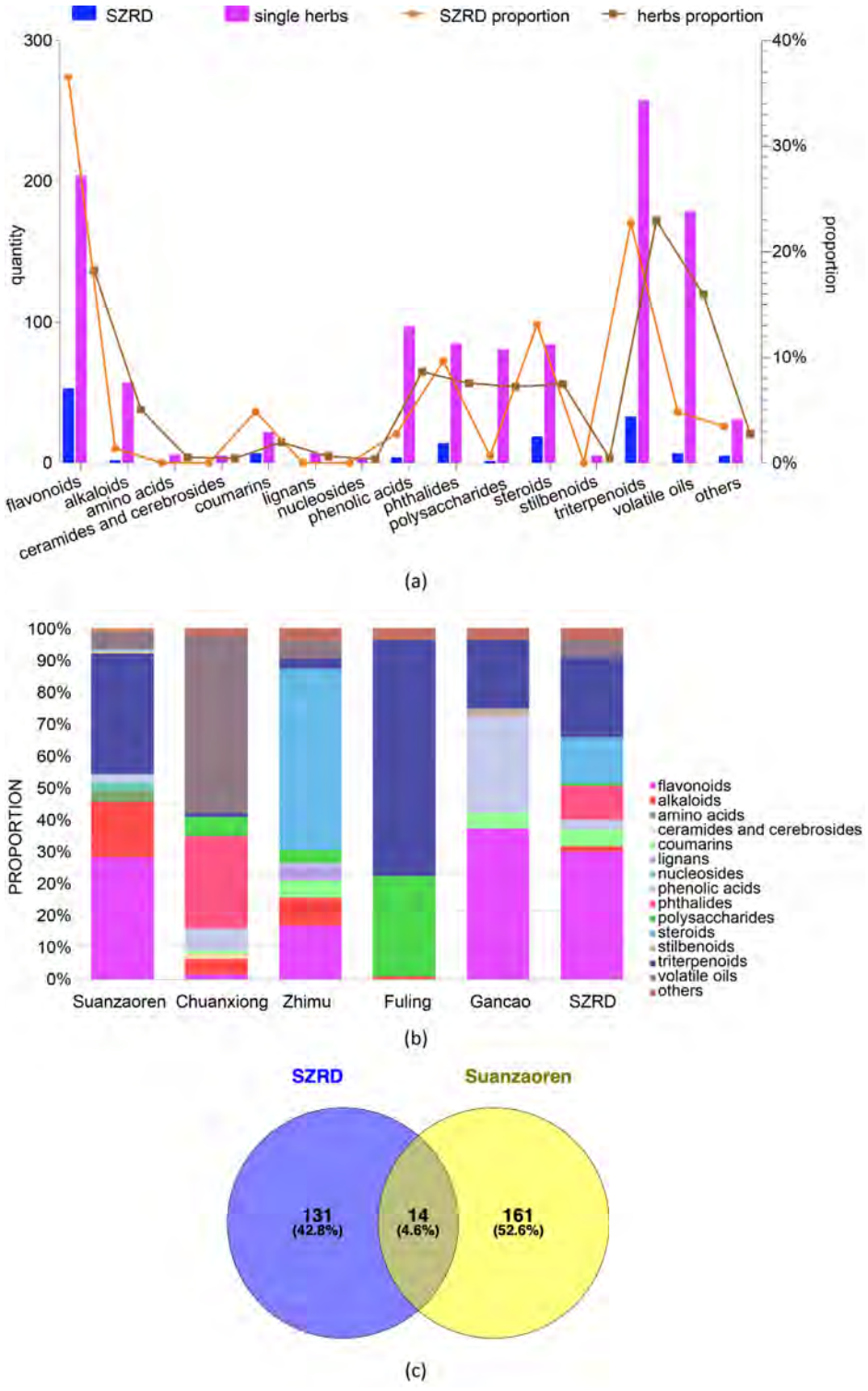

Figure 4. Chemical compounds in suanzaoren decoction and five-single herbs. (a) Proportion and quantity of various ingredients in total ingredients of herbs and SZRD. The column chart shows the quantity of various ingredients in the total compounds of herbs or the prescription, while line chart shows the proportion of various ingredients in the total compounds of herbs or the prescription. The left ordinate indicates quantity and the right ordinate indicates proportion. (b) Proportion of various ingredients in herbs and SZRD. (c) Intersection compounds of SZRD and Ziziphi Spinosae Semen.

compounds (90.3%) (Figure 4(c)), including flavonoids (45 compounds), alkaloid (1 compound), coumarins (7 compounds), phenolic acids (4 compounds), phthalides (14 compounds), triterpenoids (29 compounds), steroids (19 compounds), volatile oils (6 compounds), polysaccharide (1 compound) and others (5 compounds) (Figure 4(a) and Figure 4(b)).

5. Summary and Perspectives

In SZRD, Ziziphi Spinosae Semen is the monarch drug, Chuanxiong Rhizoma and Poria are minister drugs, Anemarrhenae rhizoma is an adjuvant, Licorice is conductant drug, which can reconcil various drugs. Totally, we collected 145 and 1104 chemical ingredients in SZRD and its five-single herbs respectively and found 80 common ingredients between SZRD and five-single herbs, while 65 different compounds only in SZRD. Taken together, our study elucidated that components of SZRD were not simply the sum of one in every single herb. Based on the analysis of the relationship of ingredients between five-single herbs and the whole prescription, we found that the problems existed in the chemical research of SZRD and possible further research direction.

5.1. What Has Already Been Shown to Be Not Promising

1) As the chemical profile of the decoction is dependent on the quality of the plant materials, each compound probably was a wide range of concentrations. Therefore, we highlighted names, numbers and variabilities of compounds in the five herbs and SZRD, nothing is said about the quantities or actual concentrations of these compounds in this review.

2) As different methods have been used, it will be difficult to compare the results of the various studies. There was an effect of the experimental conditions on the overall extracted chemical profile of the decoction. It may be different conditions and time of soaking and decocting, different extraction solvents, different apparatus and parameters to identify SZRD and five-single herbs. That means this number of compounds is very much based on the sensitivity and selectivity of the methods of analysis. For example, the compounds mentioned represent a wide range of polarities, and many of them from five-single herbs will probably not being extraction extracted in SZRD.

3) The variability in the raw materials had an effect on the plant materials on the overall extracted chemical profile of the decoction. For example, the different local growing environments of plants.

4) The different understanding of researchers on the compounds, and global chemical components had an effect on compound being obtained and identified in herbs.

5) It is unbalanced at present of the studies among Ziziphi Spinosae Semen, Chuanxiong Rhizoma, Anemarrhenae rhizoma, Poria, Licorice and SZRD, that mean there were more compounds in herbs with many reports than that with few reports. It was lack of global chemical components for each herb and SZRD.

5.2. What Looks Promising

1) As SZRD is composed of Ziziphi Spinosae Semen, Chuanxiong Rhizoma, Anemarrhenae rhizoma, Poria, Licorice, it is necessary to perform parallel studies among SZRD and its herbs.

2) As pharmacological activity has a dose-response curve, moreover synergy and antagonistic effect may occur between compounds in certain ratios, it is the biological activity of the individual compounds and their concentration that determine the activity of the final preparation. Therefore, it is necessary to study phytochemistry and pharmacological activities at the same time.

3) What is needed is to integrate the data on activity of extracts and pure compounds with the data on traditional use and the data on the chemistry.

5.3. The Limitations of This Article

1) Compounds collection of SZRD and five-single herbs were mainly based on the literature in the past five years, but few had been reported more than five years.

2) This paper did not carry out a classification analysis for non-English name compounds.

3) For the chemical composition of SZRD and its five-single herbs, we just simply classified them based on their chemical structures while not carrying out subcategory analysis.

Although these results are exploratory and need to be interpreted with caution, our study provides important information that may help offer subsequent studies to determine the compounds and explore pharmacology research about SZRD. This review may provide the reference for phytochemistry and pharmacology researches of SZRD, and also point out the direction for its further research.

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Consent for Publication

All authors have given consent for publication.

Competing interests

The authors declare that they have no competing interests.

References

[1] Li, L. (2019) Clinical Observation on Suanzaoren Decoction in Treating Insomnia. *Modern Medicine and Health Research*, **3**, 56-59.

[2] Singh, A. and Zhao, K. (2017) Treatment of Insomnia with Traditional Chinese Herbal Medicine. *International Review of Neurobiology*, **135**, 97-115.

https://doi.org/10.1016/bs.irn.2017.02.006

[3] Zhou, Q.H., Wang, H.L., Zhou, X.L., Xu, M.B., Zhang, H.F., Huang, L.B., Zheng, G.Q. and Lin, Y. (2017) Efficacy and Safety of Suanzaoren Decoction for Chronic Insomnia Disorder in Adults: Study Protocol for Randomised, Double-Blind, Double-Dummy, Placebo-Controlled Trial. *BMJ Open*, **7**, e014280. https://doi.org/10.1136/bmjopen-2016-014280

[4] Hu, K. and Hu, X. (2019) Effect of Suanzaoren Decoction on Insomnia after Stroke. *Contemporary Medical Symposium*, **17**, 200-201.

[5] Yang, T.Y., Tang, H., Qin, Y.Q. and Song, P.X. (2016) Effective Observation on Treating 26 Cases of Insomnia after Spinal Cord Injury with the Suanzaoren Decoction. *Clinical Journal of Chinese Medicine*, **8**, 79-80.

[6] Yang, Y. (2016) Effect of Suanzaoren Decoction on Hyperthyroidism and Insomnia. *Guide of China Medicine*, **14**, 213.

[7] Yeh, C.H., Arnold, C.K., Chen, Y.H. and Lai, J.N. (2011) *Suan Zao Ren Tang* as an Original Treatment for Sleep Difficulty in Climacteric Women: A Prospective Clinical Observation. *Evidence-Based Complementary and Alternative Medicine*, **2011**, Article ID: 673813. https://doi.org/10.1155/2011/673813

[8] Li, J.Y. and Wang, X. (2004) Clinical Analysis of Suanzaoren Decoction in the Treatment of Generalized Anxiety Disorder. *Journal of Shandong University of Traditional Chinese Medicine*, **28**, 438-439.

[9] Wang, Q. (2018) Effects of Addition and Subtraction Suanzaoren Decoction Treatment on Clinical Symptoms and Sleep Quality for Patients with Maintenance Hemodialysis Complicated with Depression. *Journal of Sichuan of Traditional Chinese Medicine*, **36**, 104-106.

[10] Li, D. (2018) Clinical Observation on Suanzaoren Decoction for the Treatment of 48 Cases of Menopausal Syndrome. *Journal of Clinical Medical*, **5**, 82-83.

[11] Sun, Z. and Zhang, X. (1998) Experience of Suanzaoren Decoction in Treating Intractable Premature Ventricular Contractions. *Shandong Journal of Traditional Chinese Medicine*, **17**, 112-113.

[12] Sun, H. and Yang, L. (2000) Suanzaoren Decoction in Treating Congenital Non-Hemolytic Jaundice. *Heilongjiang Journal of Traditional Chinese Medicine*, **5**, 36-37.

[13] Huang, D. and Hui, Z. (2017) SuanZaoRen Decoction Jointed with Estazolam in Treating 43 Patients with Insomnia of Liver Depression and Blood Deficiency Pattern. *Western Journal of Traditional Chinese Medicine*, **30**, 93-95.

[14] Liu, L.Y. (2018) Study on the Effect of Suanzaoren Decoction Combined with Zhizizhi Decoction on Anxiety-Induced Insomnia. *China Practical Medicine*, **13**, 138-139.

[15] Cai, C., Li, A., Zhang, R., Ma, C. and Feng, L. (2020) Effects of Suanzaoren Decoction and Sweet Dream Oral Liquid on Neuroendocrine, Cognitive Function and Deep Sleep in Patients with Sleep Disorder. *Chinese Archives of Traditional Chinese Medicine*, **38**, 210-212. http://kns.cnki.net/kcms/detail/21.1546.R.20190814.1535.071.html

[16] Li, Y. and Yuan, F. (2019) Effect of Ziziphi Spinosae Decoction Combined with Acupuncture on Insomnia and Anxiety of Uremia Patients Undergoing Maintenance Dialysis. *Journal of Liaoning University of TCM*, **21**, 133-136.

[17] Ji, S. (2019) Observation on the Effect of Suanzaoren Decoction Combined with Deanxit in the Treatment of Generalized Anxiety Disorder. *Inner Mongolia Journal of Traditional Chinese Medicine*, **38**, 48-49.

[18] Jang, X., Xu, H., Zhou, H. and Guo, Q. (2020) Suanzaoren Decoction in the Treatment of 30 Cases of Generalized Anxiety Disorder of Yin Deficiency and Fire Excitement. *Journal of North Pharmacy*, **17**, 83-84.

[19] Zhou, Z. (2016) Suanzaoren Decoction Combined with Flupentixol and Melitracen Tablets and Oryzanol in the Treatment of Perimenopausal Depression Randomized Controlled Study. *Journal of Practical Traditional Chinese Internal Medicine*, **30**, 55-57.

[20] Zhang, Y. (2017) Observation on the Curative Effect of Guipi Decoction Combined with Suanzaoren Decoction in the Treatment of Depression. *Guangming Journal of Chinese Medicine*, **32**, 512-514.

[21] Liu, Y. (2017) Clinical Observation on 36 Cases of Perimenopausal Syndrome Treated with Danzhi Xiaoyao Powder and Suanzaoren Decoction. *Hunan Journal of Traditional Chinese Medicine*, **33**, 58-60.

[22] Niu, X., He, B., Du, Y., Sui, Z., Rong, W., Wang, X., Li, Q. and Bi, K. (2018) The Investigation of Immunoprotective and Sedative Hypnotic Effect of Total Polysaccharide from Suanzaoren Decoction by Serum Metabonomics Approach. *Journal of Chromatography B: Analytical Technologies in the Biomedical and Life Sciences*, **1086**, 29-37. https://doi.org/10.1016/j.jchromb.2018.04.004

[23] Wang, D., Li, Q., Liu, R., Xu, H., Yin, Y., Wang, Y., Wang, H. and Bi, K. (2019) Quality Control of Semen Ziziphi Spinosae Standard Decoction Based on Determination of Multi-Components Using TOF-MS/MS and UPLC-PDA Technology. *Journal of Pharmaceutical Analysis*, **9**, 406-413. https://doi.org/10.1016/j.jpha.2019.01.001

[24] Zhang, F.X., Li, M. and Qiao, L.R. (2016) Rapid Characterization of Ziziphi Spinosae Semen by UPLC-Q-TOF-MS with Novel Informatics Platform and Its Application in Evaluation of Two Seeds from Ziziphus Species. *Journal of Pharmaceutical & Biomedical Analysis*, **122**, 59-80. https://doi.org/10.1016/j.jpba.2016.01.047

[25] Zhu, X., Liu, X., Pei, K., Duan, Y., Zhu, H., Ma, J., Xu, Y., Wu, Z., Zhou, Q. and Cai, B. (2018) Development of an Analytical Strategy to Identify and Classify the Global Chemical Constituents of Ziziphi Spinosae Semen by Using UHPLC with Quadrupole Time-of-Flight Mass Spectrometry Combined with Multiple Data-Processing Approaches. *Journal of Separation Science*, **41**, 3389-3396. https://doi.org/10.1002/jssc.201800171

[26] Zhao, J., Li, S.P., Yang, F.Q., Li, P. and Wang, Y.T. (2006) Simultaneous Determination of Saponins and Fatty Acids in *Ziziphus jujuba* (Suanzaoren) by High Performance Liquid Chromatography-Evaporative Light Scattering Detection and Pressurized Liquid Extraction. *Journal of Chromatography A*, **1108**, 188-194. https://doi.org/10.1016/j.chroma.2005.12.104

[27] Xie, Y.Y., Xu, Z.L., Wang, H., Kano, Y. and Yuan, D. (2011) A Novel Spinosin Derivative from Semen Ziziphi Spinosae. *Journal of Asian Natural Products Research*, **13**, 1151-1157. https://doi.org/10.1080/10286020.2011.623128

[28] Xie, J., Zhang, Y., Wang, L., Qi, W. and Zhang, M. (2012) Composition of Fatty Oils from Semen Ziziphi Spinosae and Its Cardiotonic Effect on Isolated Toad Hearts. *Natural Product Research*, **26**, 479-483. https://doi.org/10.1080/14786419.2010.516433

[29] Wang, Y., Ding, B., Luo, D., Chen, L.Y., Hou, Y.L., Dai, Y. and Yao, X.S. (2013) New Triterpene Glycosides from Ziziphi Spinosae Semen. *Fitoterapia*, **90**, 185-191. https://doi.org/10.1016/j.fitote.2013.07.013

[30] Li, M., Wang, Y., Li, C., Dai, Y. and Yao, X.S. (2014) A New Flavone C-Glycoside

from Ziziphi Spinosae Semen. *Chinese Traditional and Herbal Drugs*, **45**, 2588-2592. (In Chinese)

[31] Wu, Y., Chen, M., Du, M.B., Yue, C.H., Li, Y.Y., Zhu, M., Liu, C., Wang, D.Y., Liu, J.G. and Hu, Y.L. (2014) Chemical Constituents from the Fruit of *Ziziphus jujuba* Mill. var. *spinosa*. *Biochemical Systematics and Ecology*, **57**, 6-10. https://doi.org/10.1016/j.bse.2014.07.009

[32] Wang, Q. (2015) Research Progress on the Chemical Compositions and Application of Semen Ziziphi Spinosae. *Heilongjiang Medicine Journal*, **28**, 259-261. (In Chinese)

[33] Geng, X. and Li, T.L. (2016) Progress on Chemical Composition and Pharmacological Activities of Semen Zizyphi Spinosae. *Acta Chinese Medicine and Pharmacology*, **44**, 84-86. (In Chinese)

[34] Liu, S., Tang, Z., Cui, C., Liu, H., Liang, Y., Zhang, Y., Wang, M. and Gu, C. (2016) The Research Progress of Chemical Composition of SuanZaoRen. *Western Journal of Traditional Chinese Medicine*, **29**, 143-146. (In Chinese)

[35] Liu, X., Zhu, X., Zhu, H., Xie, L., Ma, J., Xu, Y., Zhou, Q., Wu, Z. and Cai, B. (2018) Simultaneous Quantification of Six Bioactive Components in Decoction of Ziziphi spinosae Semen Using Ultrahigh Performance Liquid Chromatography Coupled with Triple-Quadrupole Mass Spectrometry. *Journal of Analytical Methods in Chemistry*, **2018**, Article ID: 8397818. https://doi.org/10.1155/2018/8397818

[36] Mao, Y.N., Wu, W.Q., Kang, Y., Wang, F.F. and Liu, Y. (2018) Rapid Analysis of Chemical Components in Zizyphi Spinosae Semen by UHPLC-LTQ-Orbitrap-MS. *China Journal of Chinese Materia Medica*, **43**, 4884-4891. (In Chinese)

[37] Du, C.H., Cui, X.F., Pei, X.P., Yan, Y. and Qin, X.M. (2019) Research Progress on Ziziphi Spinosae Semen Saponins and Its Biological Action on Nervous System. *Chinese Traditional and Herbal Drugs*, **50**, 1258-1268. (In Chinese)

[38] Zhou, S.N., Chen, A.J., Guo, B.L., Zhang, Q.Y. and Qin, X.L. (2019) Determination of Two Flavonoids and Two Saponins in Ziziphi Spinosae Semen from Different Producing Areas. *Chinese Traditional and Herbal Drugs*, **50**, 2712-2717. (In Chinese)

[39] Chen, Y., Jiang, E., Yan, J. and Tao, Y. (2020) Validation of an Analytical Method Using UPLC-MS/MS to Quantify Four Bioactive Components in Rat Plasma and Its Application to Pharmacokinetic Study of Traditional and Dispensing Granules Decoction of *Ziziphi Spinosae Semen*. *Biomedical Chromatography*, **34**, e4797. https://doi.org/10.1002/bmc.4797

[40] Shergis, J.L., Ni, X., Sarris, J., Zhang, A.L., Guo, X., Xue, C.C., Lu, C. and Hugel, H. (2017) *Ziziphus spinosa* Seeds for Insomnia: A Review of Chemistry and Psychopharmacology. *Phytomedicine*, **34**, 38-43. https://doi.org/10.1016/j.phymed.2017.07.004

[41] Li, S., Liu, L., Pan, Y., Ao, H. and Tang, Y. (2018) GC-MS Analysis and Antioxidant Activity Study of Essential Oil from *Ligusticum chuanxiong* Hort. *Chinese Journal of Ethnomedicine and Ethnopharmacy*, **27**, 16-20. (In Chinese)

[42] Gao, X., Sun, W., Qi, L. and Li, Y. (2018) Ultra-Performance Liquid Chromatography Coupled with Electrospray Ionization/Quadrupole Time-of-Flight Mass Spectrometry for the Rapid Analysis of Constituents in Chuanxiong Rhizoma. *Northwest Pharmaceutical Journal*, **33**, 711-715. (In Chinese)

[43] Yang, J., Feng, X.L., Yu, Y., Wang, Q., Zou, J., Wang, C.X., Mu, Z.Q., Yao, X.S. and Gao, H. (2016) Novel Phthalide Derivatives Identified from *Ligusticum chuanxiong* (*Chuanxiong*). *Chinese Medicine*, **11**, Article No. 10.

https://doi.org/10.1186/s13020-016-0080-2

[44] Li, W., Tang, Y., Chen, Y. and Duan, J.A. (2012) Advances in the Chemical Analysis and Biological Activities of Chuanxiong. *Molecules*, **17**, 10614-10651. https://doi.org/10.3390/molecules170910614

[45] Zhang, H., Han, T., Yu, C.H., Jiang, Y.P., Peng, C., Ran, X. and Qin, L.P. (2012) Analysis of the Chemical Composition, Acute Toxicity and Skin Sensitivity of Essential Oil from Rhizomes of *Ligusticum chuanxiong*. *Journal of Ethnopharmacology*, **144**, 791-796. https://doi.org/10.1016/j.jep.2012.10.010

[46] Liu, Q., Zhou, J., Yu, J., Xie, Y., Jiang, X., Yang, H. and Chen, X. (2014) Systematic and Efficient Separation of 11 Compounds from Rhizoma Chuanxiong via Counter-Current Chromatography-Solid Phase Extraction-Counter-Current Chromatography Hyphenation. *Journal of Chromatography A*, **1364**, 204-213. https://doi.org/10.1016/j.chroma.2014.08.087

[47] Du, J.C., Xie, X.F., Xiong, L., Sun, C. and Peng, C. (2016) Research Progress of Chemical Constituents and Pharmacological Activities of Essential Oil of *Ligusticum chuanxiong*. *China Journal of Chinese Materia Medica*, **41**, 4328-4333. (In Chinese)

[48] Hu, J., Jia, X., Fang, X., Li, P., He, C. and Chen, M. (2016) Ultrasonic Extraction, Antioxidant and Anticancer Activities of Novel Polysaccharides from Chuanxiong Rhizome. *International Journal of Biological Macromolecules*, **85**, 277-284. https://doi.org/10.1016/j.ijbiomac.2015.12.046

[49] Li, L.J., Su, Y.F. and Yan, S.L. (2016) Three New Phthalide Glycosides from the Rhizomes of *Ligusticum chuanxiong*. *Phytochemistry Letters*, **17**, 14-17. https://doi.org/10.1016/j.phytol.2016.05.013

[50] Han, W. (2017) Advances in Chemical Constituents and Pharmacological Effects of *Ligusticum chuanxiong*. *Modern Chinese Medicine*, **19**, 1341-1349. (In Chinese)

[51] Wei, W., Xu, W. and Yang, X.W. (2017) Two New Phthalide Dimers from the Rhizomes of *Ligusticum chuanxiong*. *Journal of Asian Natural Products Research*, **19**, 704-711. https://doi.org/10.1080/10286020.2016.1275584

[52] Zhang, X., Han, B., Feng, Z., Jiang, J., Yang, Y. and Zhang, P. (2018) Bioactive Thionic Compounds and Aromatic Glycosides from *Ligusticum chuanxiong*. *Acta Pharmaceutica Sinica B*, **8**, 818-824. https://doi.org/10.1016/j.apsb.2018.04.002

[53] Zhang, X., Han, B., Feng, Z.M., Yang, Y.N., Jiang, J.S. and Zhang, P.C. (2018) Ferulic Acid Derivatives from *Ligusticum chuanxiong*. *Fitoterapia*, **125**, 147-154. https://doi.org/10.1016/j.fitote.2018.01.005

[54] Liu, J.R., Liu, Y.S., Liu, H.M., Zhang, C.Y., Wei, A.L., Ye, Q. and Guo, L. (2020) Comparison on Volatile Oil Content and Chemical Constituents among Different Parts of *Ligusticum chuanxiong* and Decoctions with Different Processing Methods. *Chinese Journal of Experimental Traditional Medical Formulae*, **26**, 101-107. (In Chinese) https://doi.org/10.13422/j.cnki.syfjx.20200314

[55] Zhang, L., Zhou, X., Lu, T.T., Mu, C.L., Wang, X.L., Tang, L.J. and Yang, R.P. (2019) Optimization of Extraction Process of Volatile Oil from *Ligusticum chuanxiong* and GC-MS Analysis of Its Overground Part. *Journal of Chinese Medicinal Materials*, **42**, 607-611. (In Chinese)

[56] Zhang, P., Chen, L., Wang, X., Chen, J., Xu, S., Ye, L. and Yao, Y. (2019) Simultaneous Determination of Night Effective Constituents and Correlation Analysis of Multiconstituents and Antiplatelet Aggregation Bioactivity *in Vitro* in Chuanxiong Rhizoma Subjected to Different Decoction Times. *Journal of Analytical Methods in Chemistry*, **2019**, Article ID: 8970624. https://doi.org/10.1155/2019/8970624

[57] Shan, L., Wu, Y., Yuan, L., Zhang, Y., Xu, Y. and Li, Y. (2017) Rapid Screening of Chemical Constituents in Rhizoma Anemarrhenae by UPLC-Q-TOF/MS Combined with Data Postprocessing Techniques. *Evidence-Based Complementary and Alternative Medicine*, **2017**, Article ID: 4032820. https://doi.org/10.1155/2017/4032820

[58] Ji, S., Su, X., Huang, Z., Su, L., Li, L. and Lu, T. (2017) Analysis of Chemical Variations between Crude and Salt-Processed *Anemarrhenae rhizoma* Using Ultra-High-Performance Liquid Chromatography-Mass Spectrometry Methods. *Molecules*, **23**, 23. https://doi.org/10.3390/molecules23010023

[59] Youn, U.J., Lee, Y.S., Jeong, H., Lee, J., Nam, J.W., Lee, Y.J., Hwang, E.S., Lee, J.H., Lee, D., Kang, S.S. and Seo, E.K. (2009) Identification of Antiadipogenic Constituents of the Rhizomes of *Anemarrhena asphodeloides*. *Journal of Natural Products*, **72**, 1895-1898. https://doi.org/10.1021/np900397f

[60] Peng, Y., Zhao, L., Lin, D.J., Liu, Y., Zhang, M. and Song, S.J. (2015) The Chemical Constituents' Determination of the Different Processed Products of *Anemarrhena asphodeloides* Rhizomes by High-Performance Liquid Chromatography Quadrupole Time-of-Flight Mass Spectrometry (HPLC-Q-TOF-MS/MS). *Biomedical Chromatography*, **30**, 508-519. https://doi.org/10.1002/bmc.3575

[61] Bak, J.P., Cho, Y.M., Kim, I., Park, D.W., Kwon, J.E., Jeong, Y.J., Kwak, J.H. and Kang, S.C. (2016) Inhibitory Effects of Norlignans Isolated from *Anemarrhena asphodeloides* on Degranulation of Rat Basophilic Leukemia-2H3 Cells. *Biomedicine & Pharmacotherapy*, **84**, 1061-1066. https://doi.org/10.1016/j.biopha.2016.10.039

[62] Nian, S., Liu, E., Fan, Y., Alolga, R.N., Li, H. and Li, P. (2016) Orthogonal Separation Protocol for the Simultaneous Preparation of four Medically Active Compounds from Anemarrhenae Rhizoma by Sequential Polyamide and Macroporous Resin Adsorbent Chromatography. *Journal of Separation Science*, **39**, 3195-3204. https://doi.org/10.1002/jssc.201600274

[63] Sun, Y., Wu, J., Sun, X., Huang, X., Li, L., Liu, Q. and Song, S. (2016) Steroids from the Rhizome of *Anemarrhena asphodeloides* and Their Cytotoxic Activities. *Bioorganic & Medicinal Chemistry Letters*, **26**, 3081-3085. https://doi.org/10.1016/j.bmcl.2016.05.012

[64] Wang, F.X., Yuan, J.C., Kang, L.P., Pang, X., Yan, R.Y., Zhao, Y., Zhang, J., Sun, X.G. and Ma, B.P. (2016) An Efficient Approach to Identify Different Chemical Markers between Fibrous Root and Rhizome of *Anemarrhena asphodeloides* by Ultra High-Performance Liquid Chromatography Quadrupole Time-of-Flight Tandem Mass Spectrometry with Multivariate Statistical Analysis. *Journal of Pharmaceutical and Biomedical Analysis*, **129**, 105-116. https://doi.org/10.1016/j.jpba.2016.06.038

[65] Yang, B.Y., Zhang, J., Liu, Y. and Kuang, H.X. (2016) Steroidal Saponins from the Rhizomes of *Anemarrhena asphodeloides*. *Molecules*, **21**, 1075. https://doi.org/10.3390/molecules21081075

[66] Jia, D., Huang, Z.Y., Fei, C.H., Xue, W.W. and Lu, T.L. (2017) Comprehensive Profiling and Characterization of Chemical Constituents of Rhizome of *Anemarrhena asphodeloides* Bge. *Journal of Chromatography B: Analytical Technologies in the Biomedical and Life Sciences*, **1060**, 355-366. https://doi.org/10.1016/j.jchromb.2017.06.032

[67] Khang, P.V., Phuong, D.M. and Ma, L. (2017) New Steroids from *Anemarrhena asphodeloides* Rhizome and Their Alpha-Glucosidase Inhibitory Activity. *Journal of Asian Natural Products Research*, **19**, 468-473. https://doi.org/10.1080/10286020.2016.1234459

[68] Sun, X.H., Zhu, F.T., Zhang, Y.W., Chen, F.F., Yu, Y., Song, N.N. and Huang, X.F. (2017) Two New Steroidal Saponins Isolated from *Anemarrhena asphodeloides*. *Chinese Journal of Natural Medicines*, **15**, 220-224. https://doi.org/10.1016/S1875-5364(17)30038-9

[69] Yang, B.Y., Bi, X.Y., Liu, Y., Li, G.Y., Yin, X. and Kuang, H.X. (2017) Four New Glycosides from the Rhizoma of *Anemarrhena asphodeloides*. *Molecules*, **22**, 1995. https://doi.org/10.3390/molecules22111995

[70] Han, F.Y., Song, X.Y., Chen, J.J., Yao, G.D. and Song, S.J. (2018) Timosaponin AIII: A Novel Potential Anti-Tumor Compound from *Anemarrhena asphodeloides*. *Steroids*, **140**, 125-130. https://doi.org/10.1016/j.steroids.2018.09.014

[71] Wang, Z., Cai, J., Fu, Q., Cheng, L., Wu, L., Zhang, W., Zhang, Y., Jin, Y. and Zhang, C. (2018) Anti-Inflammatory Activities of Compounds Isolated from the Rhizome of *Anemarrhena asphodeloides*. *Molecules*, **23**, 2631. https://doi.org/10.3390/molecules23102631

[72] Li, X., Liu, Y., Guan, W., Xia, Y., Zhou, Y., Yang, B. and Kuang, H. (2019) Physicochemical Properties and Laxative Effects of Polysaccharides from *Anemarrhena asphodeloides* Bge. in Loperamide-Induced Rats. *Journal of Ethnopharmacology*, **240**, Article ID: 111961. https://doi.org/10.1016/j.jep.2019.111961

[73] Liao, Z.D., Xu, F.Q., Wu, D.L., Zhang, W. and Huang, Q. (2019) A New Benzophenone Isolated from Fibrous Roots of *Anemarrhena asphodeloides*. *China Journal of Chinese Materia Medica*, **44**, 1392-1396. (In Chinese)

[74] Wu, D.L., Liao, Z.D., Chen, F.F., Zhang, W., Ren, Y.S., Wang, C.C., Chen, X.X., Peng, D.Y. and Kong, L.Y. (2019) Benzophenones from *Anemarrhena asphodeloides* Bge. Exhibit Anticancer Activity in HepG2 Cells via the NF-kappaB Signaling Pathway. *Molecules*, **24**, 2246. https://doi.org/10.3390/molecules24122246

[75] Zhao, Y.F., Zhou, J., Zhang, M.J., Zhang, M. and Huang, X.F. (2020) Cytotoxic Steroidal Saponins from the Rhizome of *Anemarrhena asphodeloides*. *Steroids*, **155**, Article ID: 108557. https://doi.org/10.1016/j.steroids.2019.108557

[76] Yang, B., Liu, Z., Hu, J., Lai, X. and Xia, P. (2016) Quantitative Determination of Sarsasapogenin in Rat Plasma Using Liquid Chromatography-Tandem Mass Spectrometry. *Journal of Chromatography B: Analytical Technologies in the Biomedical and Life Sciences*, **1022**, 213-219. https://doi.org/10.1016/j.jchromb.2016.04.020

[77] Zhu, L., Wang, X., Li, S., Qi, E.R., Meng, J., Ching Lam, K.Y., Dong, X., Xu, J., Chen, H. and Zhao, Z. (2020) Qualitative and Quantitative Characterization of Carbohydrate Profiles in Three Different Parts of *Poria cocos*. *Journal of Pharmaceutical and Biomedical Analysis*, **179**, Article ID: 113009. https://doi.org/10.1016/j.jpba.2019.113009

[78] Zou, Y.T., Long, F., Wu, C.Y., Zhou, J., Zhang, W., Xu, J.D., Zhang, Y.Q. and Li, S.L. (2019) A Dereplication Strategy for Identifying Triterpene Acid Analogues in *Poria cocos* by Comparing Predicted and Acquired UPLC-ESI-QTOF-MS/MS Data. *Phytochemical Analysis*, **30**, 292-310. https://doi.org/10.1002/pca.2813

[79] Zhang, G., Wang, H., Xie, W., Wang, Q., Wang, X., Wang, C., Du, Y., Huo, C. and Wang, Q. (2019) Comparison of Triterpene Compounds of Four Botanical Parts from *Poria cocos* (Schw.) Wolf Using Simultaneous Qualitative and Quantitative Method and Metabolomics Approach. *Food Research International*, **121**, 666-677. https://doi.org/10.1016/j.foodres.2018.12.036

[80] Ling, Y., Chen, M., Wang, K., Sun, Z., Li, Z., Wu, B. and Huang, C. (2012) Systematic Screening and Characterization of the Major Bioactive Components of *Poria cocos* and Their Metabolites in Rats by LC-ESI-MS^n. *Biomedical Chromatography*, **26**, 1109-1117. https://doi.org/10.1002/bmc.1756

[81] Li, S., Wang, Z., Gu, R., Zhao, Y., Huang, W., Wang, Z. and Xiao, W. (2016) A New Epidioxy-Tetracyclic Triterpenoid from *Poria cocos* Wolf. *Natural Product Research*, **30**, 1712-1717. https://doi.org/10.1080/14786419.2015.1136909

[82] Chen, T., La, H., Chou, G., Mao, X. and Zou, X. (2018) A Unique Naphthone Derivative and a Rare 4,5-Seco-Lanostane Triterpenoid from *Poria cocos*. *Molecules*, **23**, 2508. https://doi.org/10.3390/molecules23102508

[83] Chen, T., Kan, Y.J., Chou, G.X. and Zhang, C.G. (2018) A New Highly Oxygenated Pregnane and Two New 5-Hydroxymethylfurfural Derivatives from the Water Decoction of *Poria cocos*. *Journal of Asian Natural Products Research*, **20**, 1101-1107. https://doi.org/10.1080/10286020.2017.1383398

[84] Feng, G.F., Zheng, Y., Sun, Y., Liu, S., Pi, Z.F., Song, F.R. and Liu, Z.Q. (2018) A Targeted Strategy for Analyzing Untargeted Mass Spectral Data to Identify Lanostane-Type Triterpene Acids in *Poria cocos* by Integrating a Scientific Information System and Liquid Chromatography-Tandem Mass Spectrometry Combined with Ion Mobility Spectrometry. *Analytica Chimica Acta*, **1033**, 87-99. https://doi.org/10.1016/j.aca.2018.06.048

[85] Lee, S., Choi, E., Yang, S.M., Ryoo, R., Moon, E., Kim, S.H. and Kim, K.H. (2018) Bioactive Compounds from Sclerotia Extract of *Poria cocos* That Control Adipocyte and Osteoblast Differentiation. *Bioorganic Chemistry*, **81**, 27-34. https://doi.org/10.1016/j.bioorg.2018.07.031

[86] Jin, J., Zhou, R., Xie, J., Ye, H., Liang, X., Zhong, C., Shen, B., Qin, Y., Zhang, S. and Huang, L. (2019) Insights into Triterpene Acids in Fermented Mycelia of Edible Fungus *Poria cocos* by a Comparative Study. *Molecules*, **24**, 1331. https://doi.org/10.3390/molecules24071331

[87] Li, X., He, Y., Zeng, P., Liu, Y., Zhang, M., Hao, C., Wang, H., Lv, Z. and Zhang, L. (2019) Molecular Basis for *Poria cocos* Mushroom Polysaccharide Used as an Antitumour Drug in China. *Journal of Cellular and Molecular Medicine*, **23**, 4-20. https://doi.org/10.1111/jcmm.13564

[88] Pu, Y.W., Liu, Z.J., Tian, H. and Bao, Y.X. (2019) The Immunomodulatory Effect of *Poria cocos* Polysaccharides Is Mediated by the Ca^{2+}/PKC/p38/NF-κB Signaling Pathway in Macrophages. *International Immunopharmacology*, **72**, 252-257. https://doi.org/10.1016/j.intimp.2019.04.017

[89] Sun, Y. (2014) Biological Activities and Potential Health Benefits of Polysaccharides from *Poria cocos* and Their Derivatives. *International Journal of Biological Macromolecules*, **68**, 131-134. https://doi.org/10.1016/j.ijbiomac.2014.04.010

[90] Wang, W., Dong, H., Yan, R., Li, H., Li, P., Chen, P., Yang, B. and Wang, Z. (2015) Comparative Study of Lanostane-Type Triterpene Acids in Different Parts of *Poria cocos* (Schw.) Wolf by UHPLC-Fourier Transform MS and UHPLC-Triple Quadruple MS. *Journal of Pharmaceutical and Biomedical Analysis*, **102**, 203-214. https://doi.org/10.1016/j.jpba.2014.09.014

[91] Zeng, H., Liu, Q., Yu, J., Jiang, X., Wu, Z., Wang, M., Chen, M. and Chen, X. (2015) One-Step Separation of Nine Structural Analogues from *Poria cocos* (Schw.) Wolf. via Tandem High-Speed Counter-Current Chromatography. *Journal of Chromatography B: Analytical Technologies in the Biomedical and Life Sciences*, **1004**, 10-16. https://doi.org/10.1016/j.jchromb.2015.09.017

[92] Wang, N., Zhang, Y., Wang, X., Huang, X., Fei, Y., Yu, Y. and Shou, D. (2016) Antioxidant Property of Water-Soluble Polysaccharides from *Poria cocos* Wolf Using Different Extraction Methods. *International Journal of Biological Macromolecules*, **83**, 103-110. https://doi.org/10.1016/j.ijbiomac.2015.11.032

[93] Qian, Q., Zhou, N., Qi, P., Zhang, Y., Mu, X., Shi, X. and Wang, Q. (2018) A UHPLC-QTOF-MS/MS Method for the Simultaneous Determination of Eight Triterpene Compounds from *Poria cocos* (Schw.) Wolf Extract in Rat Plasma: Application to a Comparative Pharmacokinetic Study. *Journal of Chromatography B: Analytical Technologies in the Biomedical and Life Sciences*, **1102-1103**, 34-44. https://doi.org/10.1016/j.jchromb.2018.10.011

[94] Zhang, W., Chen, L., Li, P., Zhao, J. and Duan, J. (2018) Antidepressant and Immunosuppressive Activities of Two Polysaccharides from *Poria cocos* (Schw.) Wolf. *International Journal of Biological Macromolecules*, **120**, 1696-1704. https://doi.org/10.1016/j.ijbiomac.2018.09.171

[95] Zhu, L.X., Xu, J., Wang, R.J., Li, H.X., Tan, Y.Z., Chen, H.B., Dong, X.P. and Zhao, Z.Z. (2018) Correlation between Quality and Geographical Origins of *Poria cocos* Revealed by Qualitative Fingerprint Profiling and Quantitative Determination of Triterpenoid Acids. *Molecules*, **23**, 2200. https://doi.org/10.3390/molecules23092200

[96] Zhu, L., Xu, J., Zhang, S., Wang, R., Huang, Q., Chen, H., Dong, X. and Zhao, Z. (2018) Qualitatively and Quantitatively Comparing Secondary Metabolites in Three Medicinal Parts Derived from *Poria cocos* (Schw.) Wolf Using UHPLC-QTOF-MS/MS-Based Chemical Profiling. *Journal of Pharmaceutical and Biomedical Analysis*, **150**, 278-286. https://doi.org/10.1016/j.jpba.2017.11.066

[97] Li, K., Ji, S., Song, W., Kuang, Y., Lin, Y., Tang, S., Cui, Z., Qiao, X., Yu, S. and Ye, M. (2017) Glycybridins A-K, Bioactive Phenolic Compounds from *Glycyrrhiza glabra*. *Journal of Natural Products*, **80**, 334-346. https://doi.org/10.1021/acs.jnatprod.6b00783

[98] Liu, Y.Y., Yang, Y.N., Feng, Z.M., Jiang, J.S. and Zhang, P.C. (2019) Eight New Triterpenoid Saponins with Antioxidant Activity from the Roots of *Glycyrrhiza uralensis* Fisch. *Fitoterapia*, **133**, 186-192. https://doi.org/10.1016/j.fitote.2019.01.014

[99] Ji, S., Li, Z., Song, W., Wang, Y., Liang, W., Li, K., Tang, S., Wang, Q., Qiao, X., Zhou, D., Yu, S. and Ye, M. (2016) Bioactive Constituents of *Glycyrrhiza uralensis* (Licorice): Discovery of the Effective Components of a Traditional Herbal Medicine. *Journal of Natural Products*, **79**, 281-292. https://doi.org/10.1021/acs.jnatprod.5b00877

[100] Asl, M.N. and Hosseinzadeh, H. (2008) Review of Pharmacological Effects of Glycyrrhiza sp. and Its Bioactive Compounds. *Phytotherapy Research*, **22**, 709-724. https://doi.org/10.1002/ptr.2362

[101] Gou, S.H., Liu, J., He, M., Qiang, Y. and Ni, J.M. (2016) Quantification and Bio-Assay of Alpha-Glucosidase Inhibitors from the Roots of *Glycyrrhiza uralensis* Fisch. *Natural Product Research*, **30**, 2130-2134. https://doi.org/10.1080/14786419.2015.1114940

[102] Chen, J., Li, W.C. and Gu, X.L. (2017) Optimized Extraction, Preliminary Characterization, and *in Vitro* Antioxidant Activity of Polysaccharides from *Glycyrrhiza Uralensis* Fisch. *Medical Science Monitor*, **23**, 1783-1791. https://doi.org/10.12659/MSM.900471

[103] Wang, J., Li, J., Wu, X., Liu, S., Li, H. and Gao, W. (2017) Assessment of Genetic Fidelity and Composition: Mixed Elicitors Enhance Triterpenoid and Flavonoid Biosynthesis of *Glycyrrhiza uralensis* Fisch. Tissue Cultures. *Biotechnology and Applied Biochemistry*, **64**, 211-217. https://doi.org/10.1002/bab.1485

[104] Wang, X.X., Liu, G.Y., Yang, Y.F., Wu, X.W., Xu, W. and Yang, X.W. (2017) Intestinal Absorption of Triterpenoids and Flavonoids from *Glycyrrhizae radix et rhi-*

zoma in the Human Caco-2 Monolayer Cell Model. *Molecules*, **22**, 1627. https://doi.org/10.3390/molecules22101627

[105] Wong, Y.F., Cacciola, F., Fermas, S., Riga, S., James, D., Manzin, V., Bonnet, B., Marriott, P.J., Dugo, P. and Mondello, L. (2018) Untargeted Profiling of *Glycyrrhiza glabra* Extract with Comprehensive Two-Dimensional Liquid Chromatography-Mass Spectrometry Using Multi-Segmented Shift Gradients in the Second Dimension: Expanding the Metabolic Coverage. *Electrophoresis*, **39**, 1993-2000. https://doi.org/10.1002/elps.201700469

[106] Yin, L., Guan, E., Zhang, Y., Shu, Z., Wang, B., Wu, X., Chen, J., Liu, J., Fu, X., Sun, W. and Liu, M. (2018) Chemical Profile and Anti-Inflammatory Activity of Total Flavonoids from *Glycyrrhiza uralensis* Fisch. *Iranian Journal of Pharmaceutical Research*, **17**, 726-734.

[107] Frattaruolo, L., Carullo, G., Brindisi, M., Mazzotta, S., Bellissimo, L., Rago, V., Curcio, R., Dolce, V., Aiello, F. and Cappello, A.R. (2019) Antioxidant and Anti-Inflammatory Activities of Flavanones from *Glycyrrhiza glabra* L. (licorice) Leaf Phytocomplexes: Identification of Licoflavanone as a Modulator of NF-kB/MAPK Pathway. *Antioxidants* (*Basel*), **8**, 186. https://doi.org/10.3390/antiox8060186

[108] Wu, S.C., Yang, Z.Q., Liu, F., Peng, W.J., Qu, S.Q., Li, Q., Song, X.B., Zhu, K. and Shen, J.Z. (2019) Antibacterial Effect and Mode of Action of Flavonoids from Licorice against Methicillin-Resistant *Staphylococcus aureus*. *Frontiers in Microbiology*, **10**, 2489. https://doi.org/10.3389/fmicb.2019.02489

[109] Wang, C., Chen, L., Cai, Z.C., Chen, C., Liu, Z., Liu, X., Zou, L., Chen, J., Tan, M., Wei, L. and Mei, Y. (2020) Comparative Proteomic Analysis Reveals the Molecular Mechanisms Underlying the Accumulation Difference of Bioactive Constituents in *Glycyrrhiza uralensis* Fisch under Salt Stress. *Journal of Agricultural and Food Chemistry*, **68**, 1480-1493. https://doi.org/10.1021/acs.jafc.9b04887

[110] Du, Y., He, B., Li, Q., He, J., Wang, D. and Bi, K. (2017) Identification and Analysis of Chemical Constituents and Rat Serum Metabolites in Suan-Zao-Ren Granule Using Ultra High Performance Liquid Chromatography Quadrupole Time-of-Flight Mass Spectrometry Combined with Multiple Data Processing Approaches. *Journal of Separation Science*, **40**, 2914-2924. https://doi.org/10.1002/jssc.201700236

[111] Zhu, Y.X., Chen, L.L., Gong, J.R. and Wang, S.F. (2014) Identification of Constituents in Suanzaoren Tang by LC-Q-TOF-MS and LC-IT-MS. *China Journal of Chinese Materia Medica*, **39**, 457-465. (In Chinese)

[112] Yang, B., Dong, W., Zhang, A., Sun, H., Wu, F., Wang, P. and Wang, X. (2011) Ultra-Performance Liquid Chromatography Coupled with Electrospray Ionization/Quadrupole-Time-of-Flight Mass Spectrometry for Rapid Analysis of Constituents of Suanzaoren Decoction. *Journal of Separation Science*, **34**, 3208-3215. https://doi.org/10.1002/jssc.201100632

[113] Zhu, L., Wang, Z., Zhai, X., Sui, Z., Wang, D., Li, Q., Bi, K., He, B. and Wang, T. (2017) Simultaneous Quantitative Determination of 13 Active Components in the Traditional Chinese Medicinal Preparation Suanzaoren Oral Liquid by HPLC Coupled with Diode Array Detection and Evaporative Light Scattering Detection. *Journal of Separation Science*, **40**, 2320-2325. https://doi.org/10.1002/jssc.201700061

[114] Kuang, Y., He, B., Du, Y., Li, J., Wang, D., Bi, K. and Li, Q. (2016) Simultaneous Determinations of Eleven Bioactive Components in Suanzaoren Decoction Granules by High-Performance Liquid Chromatography and Its Application to the Quality Control in Productive Processes. *Analytical Sciences*, **32**, 931-936.

https://doi.org/10.2116/analsci.32.931

[115] Wang, Y., Yang, X., Xia, P.F., Ma, X., Yang, R.Q., Wang, Y.X., Fan, Q. and Zhao, L. (2020) Review of Chemical Constituents, Pharmacological Effects and Clinical Applications of Suanzaoren Decoction and Prediction and Analysis of Its Q-Marker. *China Journal of Chinese Materia Medica*, **45**, 2765-2771. (In Chinese)

[116] Bao, K.D., Li, P., Qi, L.W., Li, H.J., Yi, L., Wang, W. and Wang, Y.Q. (2009) Characterization of Flavonoid Metabolites in Rat Plasma, Urine, and Feces after Oral Administration of Semen Ziziphi Spinosae Extract by HPLC-Diode-Array Detection (DAD) and Ion-Trap Mass Spectrometry (MS). *Chemical and Pharmaceutical Bulletin*, **57**, 144-148. https://doi.org/10.1248/cpb.57.144

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Puberty, Pregnancy, Parturition, Puerperium—Surveillance by Intertwined Innumerable Neurohumoral Factors; Prevention, Postponement, Termination of Pregnancy, Precipitation of Parturition, Hysterectomy [Except for Post Partum Hemorrhage, Cancer Uteri]—Deleterious

—Proof of Basic Concept Study by Retrospective Analysis

S. Elizabeth Jeya Vardhini[1], Darmalingam[1], Celestine Raj Manohar[1], Veerakesari[1], Maharani[2], Prema[2], Sudanthira Devi[2]

[1]Department of General Medicine, Karpagam Faculty of Medical Sciences and Research, University of Madras, Tamil Nadu, India
[2]Department of Obstetrics and Gynaecology, Karpagam Faculty of Medical Sciences and Research, University of Madras, Tamil Nadu, India
Email: elizabethjsamuel@gmail.com

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Abstract

Case 1: In 1990, a 23-year-old woman, married for two years, with primary infertility, was brought by her husband, with ultrasonography of abdomen, pelvis report stating multiple tiny cysts in both ovaries, infantile uterus; so husband claimed he was cheated to marry a woman with an infantile uterus, he wanted to divorce her on medical grounds. Analysing the problem revealed the woman had irregular menstruation before marriage due to polycystic ovaries; the husband took a prescription of oral contraceptive pills from a clinician, for one cycle to regularise menstruation of his wife; which he continued to administer for 2 years, with a desire to enable conception of his wife not understanding oral contraceptive pills with their exogenous oestrogen, suppress endogenous oestrogen preventing ovulation to conceive, produce withdrawal bleeding, due to suppressed endogenous oestrogen-suppressed uterine development resulting in infantile uterus. **Case 2:** In 1996, a 25-year-old woman underwent lower segment Cesarian section, 10 days prior to her EDC [expected date of child birth], as per the request of her husband who desired to see the baby before boarding his flight overseas; lower segment Cesarian section was performed by a urologist, general surgeon, but the mother ex-

pired on the theatre table, probably because the woman's expected date of childbirth range would have fallen into the 15 days after expected date of childbirth norm, when her oestrogen would not have dipped, oxytocin would not have been released, to prevent postpartum haemorrhage. **Case 3:** In 1998, a 27-year-old woman presented with postpartum haemorrhage of one hour duration, following vaginal delivery of foeto placental unit; with haemoglobin of 3 gm%; immediately hysterectomy of the soft uterus was performed, mobilising 10 units of blood; once bleeding uterus was severed, all the 10 units of blood were transfused immediately, she survived. **Case 4:** In 1999 woman of 32 years [without antenatal screening] was delivering a twin breech presentation, she was detected to be hepatitis B surface antigen positive, she had jaundice total bilirubin 3 mg/dl, anaemia-haemoglobin of 6.5 gm%, her twins were managed at higher centres, for jaundice during pregnancy; she received 3 units of packed red blood cells, during postpartum; she returned for next pregnancy in the second trimester, with both twins crawling at her sides, Hepatitis B surface antigen had turned negative. **Case 5:** In 2003, a 32-year-old woman presented to emergency with dyspnoea with desaturation of 60%, she was ventilating but oxygen saturation was low; she had consumed hormonal pills for 3 days to postpone her menstruation to enable her to attend a wedding; she had undergone puerperal sterilisation in the past; her electrocardiography showed S1, Q3, T3 changes suggesting pulmonary embolism; thrombolysis, heparinisation was initiated, intubated, ventilated without improvement in oxygen saturation; hence saddle thrombus possibility was considered and she was referred to higher centres but she succumbed. This persons contraception status increases thrombogenicity due to reduced endogenous oestrogen status secondary to germ cells destruction by contraception, over that her consumption of hormonal pills to postpone menstruation further decreases endogenous oestrogen, increased vulnerability for pulmonary thromboembolism. **Case 6:** In 2014, a 29-year-old woman presented with unconsciousness of 30 minutes duration to emergency; she had infertility for 11 years and had delivered a precious baby, 34 days prior to admission; due to social ignorance [to enhance mothers milk baby shark food helps] she had consumed baby shark food one hour prior to admission; on examination she had quadriparesis, she was unresponsive. Her Magnetic Resonance Imaging [MRI] brain, with arteriogram, venogram showed multiple vessel narrowing suggesting vasculitis with bilateral asymmetrical, multifocal infarcts. She was treated with IV immunoglobulin, [0.4 gm/kg/day*5 days] needed ventilatory support, antiedema measures, anti-epileptics, parenteral hydration, enabling a slow recovery, on referral to higher centres.

Keywords

Endogenous Oestrogen, Pregnancy, Neuro-Humoral Factors

1. Introduction

Pregnancy, puberty, parturition, puerperium, are governed by innumerable, in-

tertwined neuro-humoral factors; comprehensive assistance to physiological parturition with monitoring, is aided by this surveillance system to marvel at. Retrospective analysis of case reports is attempted to highlight our inability to prevent morbidity, mortality brought on by precipitation of parturition, prevention, postponement, termination of pregnancy, and associated discussions in puberty, hysterectomy.

2. Case 1: Details

In 1990, a 23-year-old woman, married for two years, labourer, consuming sunflower seed oil/packed refined [ration shop] palmolein oils, with primary infertility, was brought by her husband, accompanied by many people of husband's relatives; the husband had a report of ultrasonography of abdomen pelvis, of his wife stating multiple tiny cysts in both ovaries, [polycystic ovaries] infantile uterus.

So husband claimed he was cheated to marry a woman with infantile uterus, he wanted to divorce her on medical grounds, because the ultra sonogram of abdomen, pelvis detected infantile uterus suggesting the cause for her infertility.

Analysing the problem revealed the woman had irregular menstruation before marriage due to polycystic ovaries; the husband had taken a prescription of Mala-D tablets [oral contraceptive pills] from a clinician, to administer for one cycle to regularise menstruation of his wife; which the husband continued to administer for 2 years to his wife, with a desire to enable conception of his wife; he thought if the wife gets menstruation she'll conceive

The husband did not realise he was giving cyclical Mala-D tablets [oral contraceptive pills], producing withdrawal bleeding; husband was happy that his wife was menstruating regularly, so his wife would conceive; not in the least understanding oral contraceptive pills with their exogenous oestrogen, suppress endogenous oestrogen preventing ovulation, so his wife would not conceive.

Husband was not aware that Mala-D tablets [oral contraceptive pills] would suppress endogenous oestrogen, on which every cell metabolism, **Figure 1** is dependent upon, *i.e.* cell growth, differentiation, controlled multiplication, degeneration, programmed cell death followed by new cell formation, over 48 - 72 hours [eg: brain, uterus, kidneys, heart, liver, lungs, intestines…].

In acquired suppressed endogenous oestrogen status secondary to consumption of Mala D tablets [oral contraceptive pills] for 2 years, in cell cycle, programmed cell death [apoptosis] will not be followed by due new cell formation, cell growth, differentiation in every tissue, organ, including uterus which resulted in infantile uterus, giving a basis for the husbands claim to consider divorce.

If the husband had detected polycystic ovaries by ultrasonography, as a cause for his wife's irregular menstruation, and corrected it by essential fatty acids rich diet consumption, from which cholesterol could be synthesised, be converted to endogenous oestrogen, so her menstruation would have been regularised, she would have conceived.

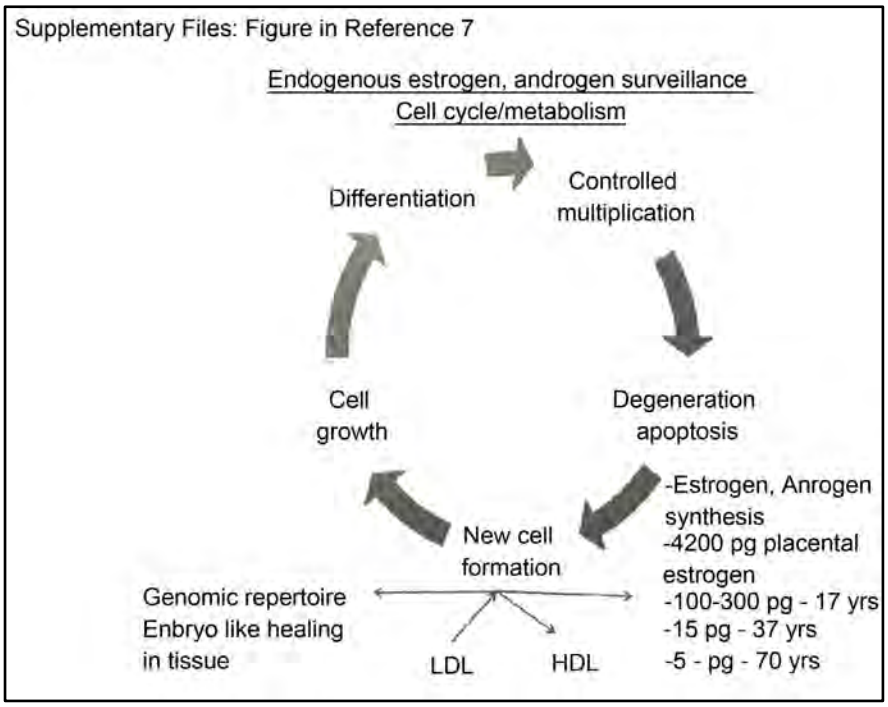

Figure 1. Cell cycle/cell metabolism/genomic repertoire, endogenous oestrogen surveillance reference [1].

3. Case 1: Discussion

At the start of each menstrual cycle, several of these ovarian follicles enlarge, usually one of the follicles in one ovary, starts to grow rapidly, to become Graafian follicle; cavity forms around ovum filled with follicular fluid; one follicle is selected to become dominant follicle in follicular phase, seems to be related [2] to the ability of the follicle to secrete oestrogen inside it, that is needed for its final maturation; primary source of circulating oestrogen is the granulosa cells of the ovaries; however the cells of theca-interna of the follicles are necessary for the production of oestrogen as they secrete androgens that are aromatised to oestrogen by the granulosa cells. 14th day of cycle, distended Graafian follicle ruptures, ovum is extruded into the abdominal cavity-called ovulation; the other follicles become atretic to become atretic follicles; this ovulation occurs under the surge of oestrogen ~300 pg/ml, under the governance of Follicular Stimulating Hormone, Luteinising Hormone of Hypothalamo pituitary axis; only if ovulation occurs around 14th day [life of ovum, 48 hours], menstruation can occur from 21st—35 days [7 days prior and 7 days after 28 days is normal for menstrual cycle].

Oestrogen levels in non pregnant women: [3].

Follicular Phase 12 - 233 pg/ml;

Ovulation phase 41 - 398 pg/ml;

Luteal phase 22 - 341 pg/ml;

Postmenopause <5 - 138 pg/ml.

Oestrogen levels in Pregnancy:

First Trimester 154 - 3243 pg/ml;

Second Trimester 1561 - 21,280 pg/ml;

Third Trimester 8525... to >30,000 pg/ml.

Oestrogen is synthesised from [4] chole sterol, [hence it gets the name steroid] which in turn is synthesised from *essential fatty acids* derived from:

1) *Edible nuts*, *seeds*, *pulses*, *legumes*, *cereals preparations*;

2) *Consumption of virgin olive oil* [highest fertility index] *virgin coconut oil*, *virgin palmolein oil* used for cooking [with life factors to harbour a seedling transferred from nuts, seeds to edible virgin oil and preparations made from nuts and seeds];

3) *Milk*, *dairy products*, [*baby's food*] *with their fatty acids*/*cream*;

4) *One cooked egg per day with its yolk,* eg: scrambled or omelette preparations [life factors contained fatty acids]; consumption of more than one egg per day produces biotin deficiency;

5) *Water living with scales gills*, *fins fish consumption supplies omega* 3, 6 *fatty acids*;

a) *Consumption of water living without gills*, *scales*, *fins representing toxin containing mammals*;

b) *Consumption of horse grams*, [meant for horse and can be metabolised by horse only, not humans];

c) C*onsumption of sea weeds* [preparations of sea weeds e.g.: custard, meant as food for water living creatures];

d) *Consumption of sprouted seeds*, *raw carrot beet root* [meant for cattle requiring renin for its digestion present in the cattle; humans have gastrin, to digest cooked beetroot, carrot, and pulses;] hence beet root, carrot, pulses, green grams need to be cooked and consumed, and not as sprouted raw seeds;

e) *Consumption of mushrooms*, toxins to our cells.

Consumption of above toxins [5] results in molecular mimicry mediated autoimmunity, leading to eg: nephritis, vasculitis, carditis, salpingitis, [resulting in tubal block] endometritis, oophoritis, pancreatitis, hepatitis, enteritis, Crohn's disease, ulcerative colitis; continuous, cumulative toxins exposure can result in metaplasia, dysplasia, neoplasms in gastrointestinal tract, hepatobiliary tree [eg: cholangio carcinoma, pancreatic cancer] with its draining lymphatics [eg: abdominal lymphoma, chronic lymphatic leukemia].

As it can be comprehended from the physiological endogenous oestrogen levels during pregnancy [~4500 - 30,000 pgm/ml], *parents life time will increase by* ~10 *years for male baby* [*xy-smaller placenta*, *smaller raw surface*], 22 *days only lochia*, *another* 22 *days for healing* [1 mm, *maximum God ordained healing capacity in the cells on all sides*], *so* 45 *days abstinence for male child delivery will suffice*; *for girl baby* [*xx*] ~20 *years increased life time for parents* [*for parents eg.*: *kidney enlarges during pregnancy*, *bones become strong every organ is strengthened by that hormonal surveillance produced by the placenta*], 45 *days lochia*, [*larger placenta larger raw detached placental surface*] *another* 45 *days*

healing, *so* 90 *days abstinence required for girl child delivery*]; similarly after menstruation starts [last menstrual period—LMP] flow for 3 days, another 3 days healing of raw endometrial surface, 7 days abstinence after last menstrual period [LMP] is required; absence of this above mentioned abstinence can result in autoimmunity [5].

When LDL, IDL contained cholesteryl esters are donated to/utilised by LDL receptors of cells to synthesise new cell membranes, steroid hormones, bile acid, HDL forms representing robust anabolic status; when cell death occurs then the cholesterol esters will be adsorbed on to HDL by LCAT [lecithin cholesterol acyl transferase], to become LDL; LDL—HDL—LDL constant metabolic process; *HDL cannot be provided by any medicine*; anabolism of new cell formation, steroid hormone synthesis, bile acid synthesis alone can generate *HDL—reflecting robust cell anabolic status—routine cells basic physiology*; cell death—cell wear and tear is accentuated by strenuous exercises like yoga, Gym, walking, jogging [detrimental] so LDL will be present, HDL levels will decrease representing more catabolism, resulting in increased degenerative changes in tissues, eg., knee joints, coronary arteries.

As per Framingham Data HDL needs to be >60 mg/dl [6] for e.g. it means if 300 cells are shed [programmed cell death—apoptosis] 300 are formed a new; [eg: the brain [6] is made up of approximately 86 billion neurons, equal numbers of neuronal, non neuronal cells make the human brain; there are approximately [7] 1 quadrillion synapses = to about half billion synapses per cubic millimeter] all have to shed and regrow every 48 - 72 hours, (genomic repertoire)—marvel of physiology requiring cholesteryl esters synthesised from essential fatty acids as listed above. This Genomic repertoire/Cell cycle/cell metabolism of cell's growth, differentiation, controlled multiplication, degeneration, programmed cell death [apoptosis], followed by new cell formation as depicted in **Figure 1**, is surveillanced by oestrogen; so cell cycle surveilling oestrogen/androgen/steroids are synthesised from cholesterol and cell membrane is also synthesised from cholesterol being life moiety of cells.

Endogenous Oestrogen enables new cell formation following apoptosis by its surveillance of cell cycle, by which it helps normal tissue development, including uterus, breasts. By oestrogen surveillance [marvel simple basic physiology] every cell physiology is protected from no differentiation, followed by uncontrolled multiplication, resulting in neoplasms.

S*o endogenous oestrogen's surveillance of cell physiology*, *serves to protect the cell from neoplasms*, [*protects from specially including breast*, *uterine cancers*] *but endogenous oestrogen enables normal tissue development*, *never will endogenous oestrogen produce cancer, endogenous oestrogen can never become carcinogenic*, [8] *to reiterate endogenous oestrogen's surveillance of normal cell cycle—cells growth differentiation*, *followed by controlled multiplication*, *degeneration*, *programmed cell death* [*apoptosis*] *followed by new cell formation is God ordained marvel physiology to protect every cell from neoplasms/cancer*

[*eg*: *endogenous oestrogen will always protect from breast cancer*].

Whereas in contraception [non evidence based non medical practice, since there is no therapeutic indication, no therapeutic protocol, no therapeutic policies, to save life from the jaws of death, diseases, rather pregnancy (life) is prevented, terminated] germ cells −20 million/day are smashed to fragmented chromatids, chromatid breaks, a-centric fragments, ring chromosomes [9] recognised as foreign leading to multi system autoimmunity; oestrogen dips to ~5 pgm/ml, [10] [androgen dips to ~1.13 ngm/ml] in contracepted parents; [11] hence every tissue follows degenerative changes.

Specially e.g. *breast dependent upon oestrogen for its normal growth*, *due to sudden*/*non physiological*/*wantonly*/*unaware acquired drop in oestrogen* [*secondary to germ cells destruction by contraception*] *attempts to mop up available oestrogen by expressing more oestrogen receptors*, *with deranged metabolism*, *due to reduced surveillance of oestrogen,* (**Figure 2**) *the cell goes for neoplasms well differentiated breast carcinoma with oestrogen receptor positivity*; *in abortions tissue damage is high*, *oestrogen surveillance* [*pregnancy-oestrogen will be high* >4500 - 30,000 pgm/ml,] *with termination of pregnancy-chaos in agonisingly reduced oestrogen surveillance-cell systems will be in highly jeopardised status*, *hence anaplastic breast carcinoma can follow*.

Only low endogenous oestrogen status, secondary to contraception [*our physiology is governed by endogenous oestrogen*, *not exogenous estrogen*], *abortion*, *sunflower seed oil*/*refined oil consumption* [*oestrogen synthesis is hampered*] *is associated with impaired surveillance of cell metabolism by reduced oestrogen status leading to neoplasms*, *including breast carcinoma*, *fibro adenoma-breast*, *leiomyoma-uterus.*

Endogenous oestrogen when it can be secreted by consumption of essential fatty acids, sufficient to reach a surge of ~300 pg/dl [under the surveillance of Follicular stimulating hormone, Leutinising hormone secreted by hypothalamo-pituitary axis] during the mid menstrual cycle, only ovulation can ensue; only

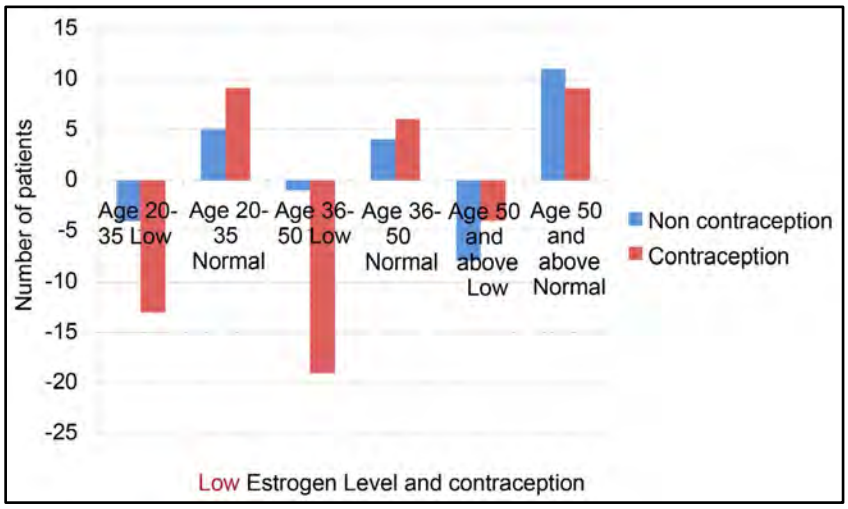

Figure 2. Reduced endogenous oestrogen levels and contraception reference [10].

then menstruation can follow on from 21st to 35 days normal menstrual cycle, provided the ovum is not fertilised; if the cyclical endogenous oestrogen, gets suppressed by exogenous oestrogen administration [as in Case 1—Mala D tablets] then surge of ~300 pg/dl of oestrogen will be lacking, leading to absent ovulation, absent maturation of Graafian follicle, several follicles which started maturing at the beginning of menstrual cycle will shrivel, ovaries will acquire many small cysts, become more poly cystic, also uterus will fail to grow normally and become infantile.

By Yoga, Gym, jogging, walking, strenuous exercises, cell death [apoptosis] programmed to occur at 48 - 72 hours, due to increased wear and tear-apoptosis [programmed cell death] gets precipitated to early e.g.: 12 - 24 hours; then degenerative changes [eg: osteoarthritis, myocardial infarction, higher incidence of spontaneous abortions, preterm delivery, cervical os laxity, by tissue damage to uterus, specially in adults practising yoga, from adolescence] will increase manifold in all tissues.

Cholesterol required for new cell membrane synthesis/steroid hormone synthesis, if not available, [due to essential fatty acids deprived diet], [oestrogen analogue consumption in yoga performers/athletes, suppresses endogenous estrogen] cell anabolism will lag behind, polycystic ovarian status is acquired.

Sunflower seed oil contains six times less vitamin E than ground nut seed oil, that small amount is also removed by refining/filtering; sunflower seed is meant for birds, we lack LDL receptors to utilise sunflower seed oil, since we are humans, resulting in impaired cell metabolism.

Cholesteryl esters synthesised from sunflower seed oil, refined oil [oil removed of its life factors contained fatty acids] transported in LDL, IDL, cannot be used by our LDL receptors, to synthesise new cell membranes, steroid hormones, [including oestrogen, progesterone, androgen] bile acids to become HDL; hence HDL levels will reduce to <45 mg/dl, resulting in 60%, 6 fold increase in degenerative/neoplastic diseases in every tissue e.g.: myocardial infarction, hemiparesis, fibroadenoma breast, leiomyoma uterus; so there'll be a metabolic defect/metabolic syndrome; LDL may be there *but HDL formation*, *will be reduced reflecting reduced status of anabolism in all tissues*.

Sunflower seed oil, [12] *refined oil consumption*, *secondary to impaired/inadequate synthesis of oestrogen*, *androgen leads to* 60%, 6 *fold degenerative changes in every tissue*, *neoplasms in any tissue e.g*: *leiomyoma-uterus*, *fibroadenoma breast*, *impaired secondary sexual dimorphism*[*normally shoulders broad for men*, *broad gynaecoid pelvis with narrow waist*, *for women*, *so for e.g.*: *the baby's head/vertex in uterus will not incur cephalo-pelvic disproportion resulting in Lower segment caesarian section*] *resulting in obesity*, *more frequent cephalo pelvic disproportion resulting in frequent lower segment Caesarian section.*

Cylindrical obesity suggests-impaired surveillance of androgen, oestrogen on secondary sexual dimorphism; reduced endogenous oestrogen/androgen status, secondary to 1) Reduced synthesis, as in consumption of sunflower seed oil/

refined oil [oil removed of its life factors contained fatty acids]; 2) Contraception, abortion secondary to germ cells/tissue destruction [agonizingly reduced endogenous oestrogen, androgen] (**Figure 2**).

Hence obesity will resolve by reversal of contraception, [*if present*] *consumption of virgin olive oil*/ *virgin coconut oil*/*virgin palmolein oil*, *for cooking*, *consumption of fried preparations made from pulses* [*by restored endogenous oestrogen*/*androgen status*] *as a cause and effect phenomenon and not by walking*, *jogging*, *gym*, *yoga*; *unchecked obesity after contraception can lead to obstructive sleep apnoea. Global many children per family norms will eradicate prevalent obesity and restore physiological secondary sexual dimorphism* [*slim*/*strong*/*healthy beauty*].

HDL < 45 mg/dl, it denotes programmed cell death, apoptosis is not replaced adequately by new cell membrane synthesis, steroid hormone synthesis, bile acid synthesis, utilising cholesterol esters of LDL, IDL, suggesting poor anabolic status, degenerative changes in every tissue will ensue, including uterus, ovaries, as in sunflower seed oil/refined oil consumption with consequently reduced oestrogen synthesis, surge of mid menstrual cycle ~300 pg/dl of oestrogen would not occur affecting Graafian follicle maturation, there may be no ovulation, so proliferative phase will be prolonged due to lack of oestrogen surge, several follicles which have attempted to mature will start shrivelling and become polycysts of ovary resulting in poycystic ovary, irregular menstruation, as in Case 1.

In utero one cell embryo becomes 2, 4, 8, 16 cells, morula stage then cells break up one cell lineage differentiates into e.g.: cardiovascular system, another cell lineage differentiates into musculoskeletal system, to become 3 kg baby, 3/4 kg placenta, under surveillance of endogenous oestrogen secreted by corpus luteum, placenta, synthesised from mothers' consumed essential fatty acids containing diet as depicted earlier; unless placenta is able to secrete in second trimester 1561 - 21,280 pg/ml, [due to sunflower seed oil/refined oil consumption] there'll be foetal demise/spontaneous abortion, due to placental switch over insufficiency, in 3 - 4 months of pregnancy.

When as in case 1, due to essential fatty acids decreased diet, [eg: sunflower seed oil, refined oil for cooking] cholesterol synthesis to be converted to endogenous oestrogen, to support ovulation/secondary sexual dimorphism results in polycystic ovaries, with associated irregular menstruation, obesity; so *metformin*, *exogenous oestrogen administration* [*Mala D*/*oral contraceptive pills*] *have no role in polycystic ovaries*; prescription of isoflavanoids, mandatory essential fatty acids consumption will rectify polycystic ovaries.

Persons on essential fatty acids deprived diet[sunflower seed oil, refined oil] will have impaired cyclical endogenous oestrogen resulting in probable delayed puberty, poly cystic ovaries, with associated irregular menstruation, obesity; difficulty to conceive, higher miscarriages/spontaneous abortions/still births, increased cephalopelvic disproportion resulting in higher incidence of lower caesarian segment section [oestrogen derived from cholesterol governs secondary sexual dimorphism].

4. Case 2: Details

In 1996, a 25-year-old woman, on regular antenatal care surveillance, approached for possibilities of considering elective lower caesarian segment section, 10days earlier, than her EDC, expected date of childbirth, to enable her husband to visualise the baby before his imminent departure to overseas.

Elective Lower Cesarian segment section was performed by her regular antenatal care providers, a surgeon, urologist, baby was delivered, the mother expired due to postpartum haemorrhage, inspite of all possible salvaging measures. Husband told if medical advise had been portrayed about possibility of maternal mortality, he would have left overseas, seen baby's photo.

Delivery 15 days prior or 15 days after EDC, expected date of childbirth is considered full term delivery; probably because the woman's expected date of childbirth range would have fallen into the 15 days after EDC norm, when her oestrogen would not have dipped, oxytocin would not have been released, to enable involution of uterus to prevent postpartum haemorrhage.

5. Case 2: Discussion

Precursor of all steroids [13] is cholesterol, most of it is taken up from LDL in the circulation by LDL receptors; cholesterol is esterified, to be followed by dehydrogenation to form progesterone.

Progesterone is synthesised [14] by the ovaries after ovulation; if fertilisation of ovum occurs, then progesterone levels rise slowly from 9th week of pregnancy until 32nd week; placenta synthesises progesterone after 12 weeks of pregnancy.

Progesterone Levels:

Luteal phase of menstrual cycle: 2 - 25 ng/ml;

First trimester of pregnancy: 10 - 44 ng/ml;

Second trimester of pregnancy: 19.5 - 82.5 ng/ml;

Third trimester of pregnancy: 65 - 290 ng/ml.

Reduced progesterone levels during pregnancy, suggests possibility of spontaneous abortions [miscarriages].

Probabilities are as endogenous oestrogen/progesterone decreases from Third Trimester 8525... to > 30,000 pg/ml, 290 ng/ml respectively, as foetus matures, oxytocin will be released from hypothalamo pituitary axis to initiate parturition, uterine contractions, establish lactation.

In pregnancy there is a dynamic balance between the forces that cause uterine quiescence and the forces that produce coordinated uterine contractility. There is also a balance between the forces that keep the cervix closed to prevent uterine emptying and the forces that soften the cervix and allow it to dilate. For delivery to occur both balances must be guided in favour of active uterine emptying. Many of the elements in this parturition complex elaborate feed forward characteristics. Labour at term is physiologically regarded as a release from the inhibitory effects of pregnancy on myometrium. Human labour at term is a multifac-

torial physiologic event involving integrity of complementary endocrine, paracrine, and autocrine factors leading to gradual changes within maternal uterine tissues [myometrium, decidua, cervix].

For parturition to occur two changes must take place in a woman's reproductive tract. First the uterus must be converted from a quiescent structure with dyssynchronous contractions to an active coordinately contracting organ with complex interlaced muscular components resulting in regular phasic uterine contractions. This requires the formation of gap junctions between myometrial cells to allow for transmission of the contractile signal. The foetus may coordinate this switch in myometrial activity through its influence on placental steroid hormone production, through the mechanical distension of the uterus and through the secretions neurohyprophseal hormones and other stimulators of prostaglandin synthesis.

The second change is that the cervical connective tissue and smooth muscle must be capable of dilatation to allow the passage of fetus from the uterus. These changes are accompanied by shift from progesterone to oestrogen dominance, increased responsiveness to oxytocin by means of up regulation of myometrial oxytocin receptor, increased Prostaglandins synthesis in uterus, increased myometrial gap junction formation, decreased nitric oxide activity and increased influx of calcium into myocytes, with ATP dependent binding of myosin to actin, increased endothelia leading to augmented uterine blood flow and myometrial activity.

The final common pathway toward labour appears to be the activation of the foetal Hypothalamo pituitary axis and is probably common to all viviparous species [15] when conception occurs safeguarded delivery of the baby becomes therapeutic indication as dictated by foetal hypothalamic pituitary axis; our efforts to assist this physiology.

Complimentary changes in the cervix involving a decrease in progesterone dominance and the actions of prostaglandins and relaxin, via connective tissue alterations, collagenolysis, and a decrease in collagen stabilization through metalloproteinase inhibitor lead to cervical softening and dilatation.

The balance between the effects of oestrogen and progesterone is critical to maintenance of pregnancy and the onset of labor; other important hormonal factors modulate this balance.

There was no therapeutic indication for lower cesarian segment section; any therapy would have therapeutic indication, therapeutic protocols, therapeutic policies, evidence based medical practice; nothing else can spell therapeutic intervention. [as the father wanted to see the baby before boarding the flight]; as the presenting foetal part descends [cephalic/breech] with effacement, dilatation of cervix, uterine contractions, with frequent foetal heart, maternal vitals monitoring labour results in normal delivery or the needful intervention. For example in myoma uterus, there is therapeutic indication for myomectomy.

Preterm [<37 weeks gestation] and post term birth [>42 weeks gestation] are

associated with increased morbidity [16] and mortality for mother and infant, eg termination of pregnancy for hypertension, renal disease [*it is not removing a cup and emptying a bucket, believing similarly for lower caesarian segment sections to terminate pregnancy for medical reasons*] support pregnancy, with antihypertensives, because oestrogen would not have dipped [blood would be hypocoagulable], oxytocin would not have been released to establish parturition, involution of uterus, to contract on bleeding vessels; so maternal postpartum mortality is inevitable, foetal survival is difficult.

Post delivery proteinuria, renal function all improve remarkably, [17] jaundice during pregnancy will abate after placental delivery; pregnancy is tuned by innumerable physiological factors guiding to labour at term.

It is the insufficient synthesis in 4500 pg/dl - 30,000 pg/dl to support foetal growth from 3rd month to term [because sunflower seed oil/refined oil consumption—oestrogen progesterone cannot be synthesised because cholesterol esters of sunflower seed oil without corresponding LDL receptors in human body cannot be utilised to synthesise steroid hormones] placenta lakes of blood with slow flowing blood will clot [because oestrogen synthesis is inadequate leading to placental infarctions resulting in eclampsia, hypertension, foetal miscarriages.

High optimal levels of oestrogen in trimesters, [by consumption of virgin palmolein oil, virgin coconut oil, virgin olive oil, fried preparations of pulses] achieves by its hyaocoagulability good placental blood flow, materno-foetal exchanges no placental infarcts, foetal growth optimal, placental switchover adequate.

Hence every human being, including mothers' consumption of virgin olive oil, virgin coconut oil, virgin palmolein oil [and not sunflower seed oil, refined oils/ packet oils] essential to prevent 60% degenerative, neoplastic, probably autoimmune diseases, in all tissues specially placental switchover insufficiency, spontaneous abortions, eclampsia, hypertension of pregnancy, cephalopelvic disproportions requiring lower cesarian segment sections at term.

Its marvellous tissue changes, under paracrine, eccrine, endocrine governances ultimately common pathway by foetal HPA axis activation as portrayed above, [foetal maturity guides to labour during which cervical os has to open, labour has to progress;] in this above case pregnancy was terminated only 10 days before her expected date of childbirth without labour pain onset, to prime the cervix, probably the mothers expected date of childbirth, was in the latter half-15 days after expected date of childbirth range, so oestrogen had not dipped [blood will be hypocoagulable to maintain flourishing flow to slow blood flowing placenta], oxytocin would not have been released, to establish parturition-uterine coordinated contractions, lactation, uterus would not involute to our intervention by LSCS, mothers mortality by postpartum haemorrhage is inevitable.

At parturition, oestrogen would have dipped, [coagualbility of blood increases with placental delivery, placental hormones would drop] oxytocin is released,

parturition with coordinated uterine contractions, softening of cervix, dilatation of cervical os with head/vertex descent, [to name a few of innumerable factors governing physiological pregnancy, physiological parturition] on foeto-placental delivery, involution of uterus, clamming down on the bleeding vessels, clotting of bleeding vessels from detached placental surface will follow because oestrogen has dipped, mother is protected from postpartum haemorrhage by God ordained physiology; again to marvel at physiology and assist only delivery, pregnancy is geared physiologically to support foetal growth to parturition at term; LSCS/ termination of pregnancy anytime believing we're removing a cup, emptying a bucket deleterious for mother, foetus; pregnancy has to be nurtured, continued with virgin olive oil, virgin coconut oil, virgin palmolein oil consumption and not refined oil or sunflower seed oil; avoiding ingested toxins, namely water living without scales gills, fins; horse grams, seaweeds, sprouted seeds, mushrooms will safe guard us, including pregnant mothers from kideney disease, proteinuria, liver disease, hepatitis B surface antigen, hepatitis C virus, [water living without scales, gills, fins are toxin containing mammals swallowing blood etc. in waters, which through their bone prick…we can contract these viruses] Gastro intestinal tract neoplasms, abdominal lymphomas, carditis, dermatitis, autoimmune diseases by molecular mimicry mediated autoimmunity [18] (**Figure 3** and **Figure 4**).

In leiomyoma there is a therapeutic indication for myomectomy and not hysterectomy [the mother wants hysterectomy…] leiomyoma occurs secondary to decrease in endogenous oestrogen status governng cell metabolism, leading to whorls of smooth muscle cells as myoma, instead of growing normally as uterus; reduced endogenous oestrogen status follows 60% with essential fatty acids deprived diet (eg. sunflower seed oil/refined oil—oil removed of its life factors

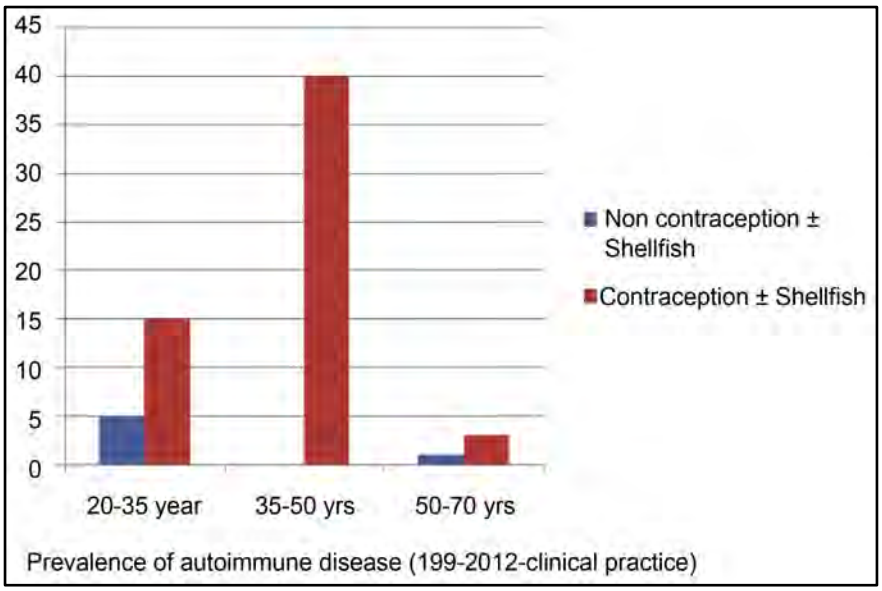

Figure 3. Prevalence of autoimmune diseases, contraception, water living without scales ingestion reference [18].

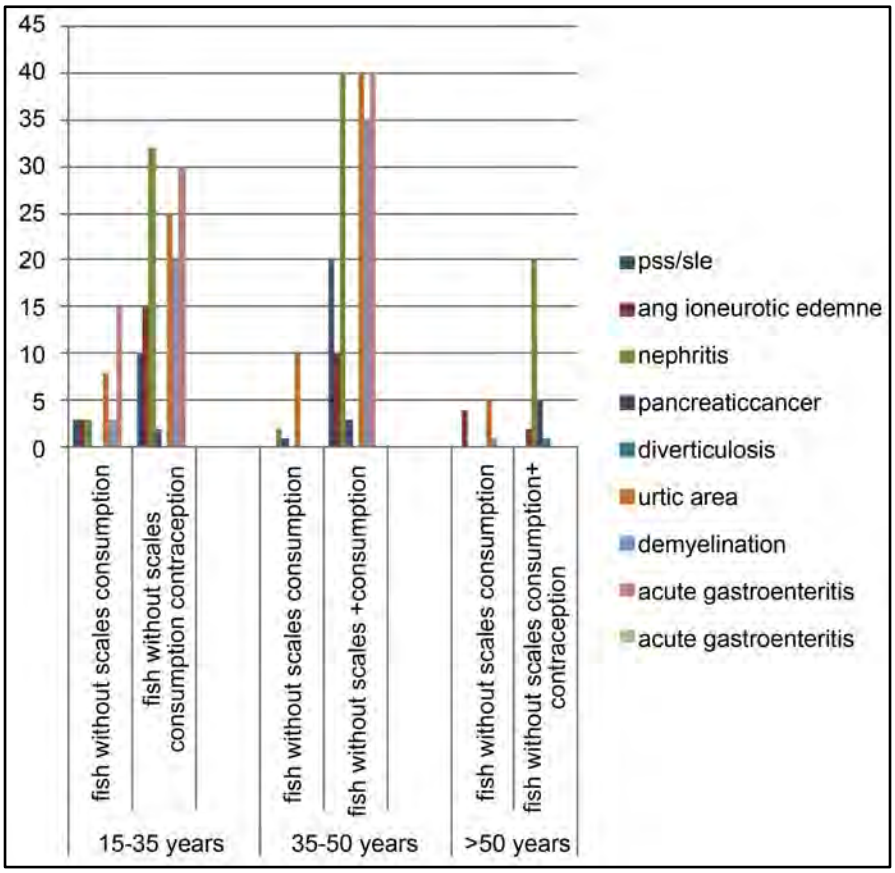

Figure 4. Prevalence of autoimmune diseases with contraception, toxinous food ingestion reference [18].

contained fatty acids consumption), oestrogen synthesis reduces, 275% after contraception/abortion [non evidence based non medical practice] secondary to fragmentation of germ cells; *hence myomectomy coupled with fallopian tubal recanalisation/contraception reversal* [*if needed*], *consumption of virgin olive oil, virgin coconut oil/virgin palmolein oil will prevent recurrence of leiomyoma.*

Similarly in uterine descent secondary to degenerative changes in pelvic floor, consequent to reduced endogenous oestrogen status governing cell metabolism [275% after contraception abortion, 60% with essential fatty acids deprived diet], *pelvic floor repair, sling procedure, ligaments plication, coupled with contraception reversal/eg. fallopian tubal recanalisation*, [*if required*] *consumption of virgin coconut oil/virgin palmolein oil/ virgin olive oil, will prevent further recurrence of uterine descent would be the protocol and not hysterectomy*; [in intestinal descent hernia we strengthen parietal abdominal wall, replace intestines, herniorraphy similarly in uterine descent]; *in hernia to prevent recurrence of degenerative changes in abdominal wall, coupled fallopian tubal recanalisation/contraception reversal*, [*if needed*] *virgin coconut oil/olive oil/palmolein oil consumption will prevent recurrence of hernia.*

In hysterectomy endogenous oestrogen dips to 0.4 pg/ml resulting in 500% increase in degenerative/neoplastic/autoimmune diseases (**Figure 5**) in both

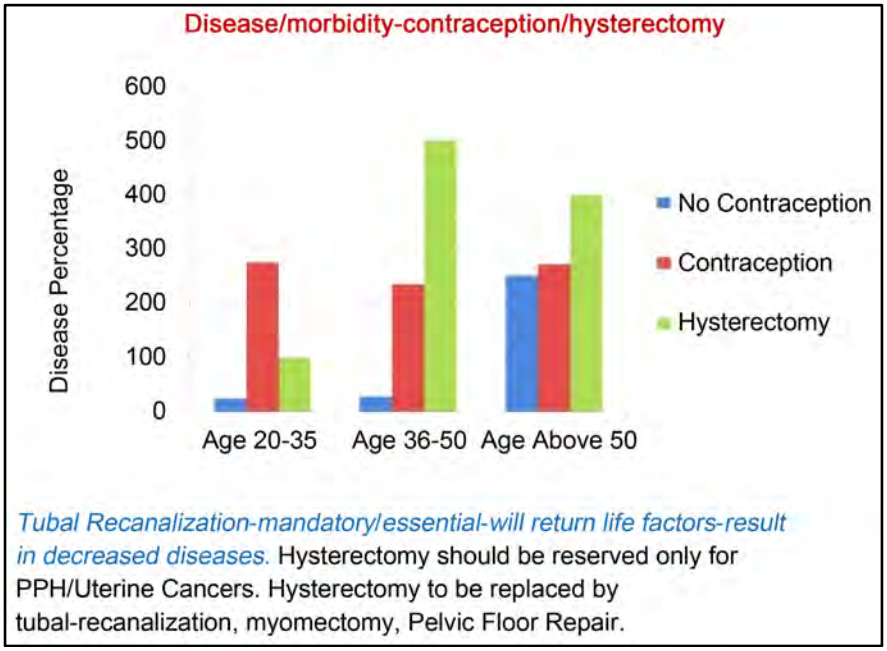

Figure 5. Morbidity prevalence with hysterectomy, contraception reference [19].

parents [19].

Conservation of life saving procedure urgent hysterectomy for cancer uterus, post-partum haemorrhage would be therapeutic protocol.

6. Case 3: Case Details

In 1998, a 27-year-old woman presented with postpartum haemorrhage of one hour duration, after prolonged labour: her haemoglobin was 3 gm%; she was on continuous bladder drainage, oxytocin infusion was going, but the uterus had not become rock hard, it remained soft; mobilising 10 units of blood, with preanesthetic panel prepared, emergency hysterectomy of the soft uterus was performed, once uterine bleeding vessels were ligated, uterus was severed, all the 10 units of blood were transfused immediately, she survived.

7. Case 3: Discussion

After vaginal delivery, after prolonged labour the lady presented with postpartum haemorrhage; after confirming uterus is soft, not rock hard inspite of oxytocin infusion, with preanesthetic panel made ready, emergency life saving hysterectomy was performed; simultaneously mobilising 10 units of blood which were transfused soon after ligation of uterine blood vessels, severing uterus. Patient survived.

Due to nitric oxide vasodilatation effect of oestrogen, pregnancy is a hypo coagulable status, hypovolemic because significant vascular volume is in third space eg. placenta; puerperium oestrogen has dipped, so its highly hypercoaguable status, prone for superior sagittal vein thrombosis, cortical vein thrombosis, if associated with dehydration; so sufficient hydration to be taken care of during

post partum; at puerperium prothrombin time prolonged status due to jaundice during pregnancy which will abate normally at delivery of placenta, not be treated with fresh frozen plasma, can precipitate superior sagittal venous thrombosis; similarly no need for Injection vitamin K for prolonged prothrombin time during pregnancy, which is physiological due to hypo coagulable status, injection vitamin K will lead to placental infarctions and foetal miscarriages.

8. Case 4: Details

In 1999 woman of 32 years [without prior antenatal screening] was delivering a twin breech presentation; she was detected to be hepatitis B surface antigen positive, she had jaundice, total bilirubin 3 mg/dl, anaemia-haemoglobin of 6.5 gm%; after coming head forceps was applied to assist delivery.

Her twins were managed at higher centres, due to jaundice, hepatitis B surface antigen positivity for mother during pregnancy; she received 3 units of packed red blood cells, during postpartum; her jaundice decreased after delivery of placenta, she returned for next pregnancy in the second trimester, with both twins crawling at her sides, Hepatitis B surface antigen had turned negative for the mother.

9. Case 4: Discussion

Pregnancy with high oestrogen status ranging about >4500 pgm in the second trimester with resultant robust cell anabolism/immunity in mother, could expel Hepatitis B virus.

10. Case 5: Details

In 2003, a 32-year-old woman presented to emergency with dyspnoea, desaturation of 60%, she was ventilating but oxygen saturation was low; prior to admission, she had consumed hormonal pills for 3 days to postpone her menstruation to enable her to attend a wedding; she had undergone puerperal sterilisation in the past; her electrocardiography showed S1, Q3, T3 changes suggesting pulmonary embolism; thrombolysis, heparinisation was initiated, intubated, ventilated without improvement in oxygen saturation; hence saddle thrombus possibility was considered and she was referred to higher centres for CT pulmonary Angiogram, Thromboembolectomy but she succumbed.

11. Case 5: Discussion

This person's contraception status, puerperal sterilisation, increases thrombogenicity [275%] due to reduced endogenous oestrogen status secondary to germ cells destruction by contraception, over that her consumption of hormonal pills to postpone menstruation further decreases endogenous oestrogen, increased vulnerability for pulmonary thromboembolism. Information, side effects regarding pills, mentions pulmonary embolism but the common public least do they comprehend pulmonary embolism could mean sudden death; she had tra-

velled to attend the wedding dehydration adding on to thromogenecity.

12. Case 6: Details

In 2014, a 29-year-old woman presented with unconsciousness of 30 minutes duration to emergency; she had infertility for 11 years and had delivered a precious baby, 34 days prior to admission; [due to social ignorance to enhance mothers milk, baby shark food helps] she had received baby shark food one hour prior to admission; on examination she had quadriparesis, she was unresponsive pupils were reacting.

Her Magnetic Resonance Imaging [MRI] brain, with arteriogram, venogram showed multiple vessel narrowing suggesting vasculitis with bilateral asymmetrical, multifocal infarcts. She was treated with IV immunoglobulin, [0.4 gm/kg/day*5days] needed ventilatory support, anti-oedema measures, anti epileptics, parenteral hydration, she was referred to higher centres.

13. Case 6: Discussion

Shark is a toxin containing mammal, as the toxins mediate molecular mediated autoimmunity, resulting in multifocal bilateral asymmetrical multiple vessel narrowing suggesting vasculitis; awareness about food toxins as already mentioned leading to autoimmunity, would have helped prevent this presentation.

14. Conclusions

Consumption of virgin olive oil, virgin coconut oil, virgin palmolein oil [essential fatty acids rich diet] for cooking can prevent polycystic ovaries, pre-eclampsia, miscarriages, cylindrical obesity, cephalopelvic disproportions.

Awareness to avoid food toxins-[waterliving without scales, horse grams, seaweeds, sprouted seeds, consumption of raw carrot, beetroot, mushrooms] can prevent toxins associated with vasculitis, nephritis, psoriasis/cutaneous lesions, hepatopathy, pancreatitis, chronic pulmonary changes, abdominal lymphoma, chronic lymphatic leukaemia, gastrointestinal neoplasms, including cholangiocarcinoma, pancreatic cancers, hepatocellular carcinoma.

Endogenous oestrogen surveillance of cell metabolism [physiology] will always protect the cells including breast, uterus from neoplasms; endogenous oestrogen can never become carcinogenic, by marvel unfailing physiology; its impaired oestrogen surveillance secondary to consumption of sunflower seed oil, refined oils, essential fatty acids deprived diet [60%], contraception abortion [275%] results in degenerative diseases, neoplasms.

Therapeutic indication following conception is delivery assisted by therapeutic protocols, policies because foetal hypothalamus pituitary axis has to coordinate innumerable paracrine, eccrine, endocrine, factors for the final common pathway of uterine emptying, for eg. oxytocin has to be released from maternal hypothalamus pituitary axis, even as oestrogen dips nearing parturition; prevention, postponement, termination of pregnancy, precipitation of par-

turition, [jumping off cliff to fly marred science].

Emergency hysterectomy with preanesthetic panel workup, blood transfusions, given after ligation of uterine vessels, severing the soft uterus, life-saving in postpartum haemorrhage following vaginal delivery of foetus, placenta.

Uterine Leiomyoma suggested therapeutic protocol-myomectomy, coupled with contraception reversal [eg. fallopian tubal recanalisation] if present, virgin coconut oil consumption to prevent further recurrence of leiomyoma; similarly, uterine descent suggested therapeutic protocol is pelvic floor repair with sling procedure/ligaments plication, coupled with contraception reversal, virgin coconut oil consumption to prevent a recurrence. Conservation of life-saving procedure urgent hysterectomy for cancer uterus, post-partum haemorrhage would be suggested therapeutic protocol.

Pregnancy with endogenous oestrogen ranging from >4500 pg/dl to >30,000 pg/dl, associated robust anabolism/immunity, enabled the elimination of hepatitis B positivity from the serum of mother.

Conflicts of Interest

The authors declare no conflicts of interest regarding the publication of this paper.

References

[1] Samuel, E., Darmalingam, Natarajan, S., Ramalingam, Manohar, C., Veerakesari, S., Kanthan, U. and Kanagaraj, C. (2019) Empty Sella [Presenting as Hypopituitarism] and Platybasia Secondary to Essential Fatty Acids Deprived Diet. Proof of Basic Concept Study by Retrospective Analysis. *Open Journal of Preventive Medicine*, **9**, 95-104. https://doi.org/10.4236/ojpm.2019.98009

[2] Barrett, K.E., Barman, S.M., Boitano, S., Brooks, H.L. (2012) Ganong's Review of Medical Physiology. 24th Edition, McGraw-Hill Education, New York, 401.

[3] US National Library of Medicine Medline Plus (2021) Estradiol (E2)-UI Health Care.

[4] Carn, B.R. and Wilson, J.D. (1987) Disorders of the Ovary and Female Reproductive tract, Cholesterol Is Essential. In: Larry Jameson, J., Fauci, A.S., Kasper, D.L., Hauser, S.L., Longo, D.L. and Loscalzo, J., Eds., *Harrison's Principles of Internal Medicine*, Vol. 2, McGraw-Hill Education, New York, 1820-1821.

[5] Samuel, E.J.V., Ramalingam, Zumu, Aakash, Rajmanohar, C., Darmalingam, Veerakesari, S. and Natarajan, S. (2020) Autoimmune Thrombotic Thrombocytopenic Purpura, Disseminated Intravascular Coagulation, Haemophagocytic Lympho Histiocytosis—(Following Corona Virus Infection), Secondary to Fragmented Germ Cells {Liberal Global Abortions, Contraception—(Red Riverways, Blood Moon, Weakened Earth's Magnet)} and Absence of Abstinence—Retrospective Analysis: Proof of Concept Study. *Open Journal of Rheumatology and Autoimmune Diseases*, **10**, 132-145. https://doi.org/10.4236/ojra.2020.103015

[6] Brown, M.S. and Goldstein, J.L. (1985) Exogenous Pathway, Endogenous Pathway. In: Gilman, A.G., Ed., *Goodman's and Gilman's Pharmacological Basis of Therapeutics*, 7th Edition, Macmillan Publishing Company, New York, 827-828.

[7] Azevedo, F.A., Carvalho, L.R., Grinberg, L.T., Farfel, J.M., Ferretti, R.E., Leite, R.E.,

Filho, W.J., Lent, R. and Herculano-Houzel, S. (2009) Equal Numbers of Neuronal and Nonneuronal Cells Make the Human Brain an Isometrically Scaled-Up Primate Brain. *Journal of Comparative Neurology*, **513**, 532-541. https://doi.org/10.1002/cne.21974

[8] Elizabeth, J.S. (2014) Cancer and Germ Cells. *Open Journal of Preventive Medicine*, **4**, 606-615. https://doi.org/10.4236/ojpm.2014.47070

[9] Gilliland, B.C. (1987) Progressive Systemic Sclerosis. In: In: Larry Jameson, J., Fauci, A.S., Kasper, D.L., Hauser, S.L., Longo, D.L. and Loscalzo, J., Eds., *Harrison's Principles of Internal Medicine*, 11th Edition, Vol. 2, McGraw Hill Lange, New York, 1429.

[10] Samuel, E.J. (2012) Increased Prevalence of Neuroleptic Malignant Syndrome, Dementia and Fragmented Germ Cells with Reduced Endogenous Estrogen. *International Journal of Science and Research* (*IJSR*), **3**, 1694-1700.

[11] Vardhini Samuel, E., Natarajan, N. and Kumar, S. (2015) "Withered Tree" Concept Host Achieved by Global Contraception, Abortion, Secondary to Fragmented Germ Cells, Agonizingly Reduced Endogenous Estrogen/Androgen, Defaulted Genomic Repertoire, Deranged Cell Metabolism, Metabolic Syndrome, Result in Failure of Pharmacological Therapeutics with Advanced Technologies. *Open Journal of Preventive Medicine*, **5**, 186-195. https://doi.org/10.4236/ojpm.2015.54022

[12] Elizabeth, J.S. (2014) Increased Prevalence of Obesity and Fragmented Germ Cells with Reduced Endogenous Oestrogen, Androgen. *International Journal of Scientific and Research Publications*, **4**, 1-5.

[13] Barrett, K.E., Barman, S.M., Boitano, S. and Brooks, H.L. (2012) Steroid Biosynthesis. In: Barrett, K.E., Boitano, S. and Barman, S.M., Eds., *Ganong's Review of Medical Physiology*, 24th Edition, McGraw Hill Lange, New York, 360.

[14] University of Rochester (2021) Progesterone-Health.

[15] Kota, S.K., Gayatri, K., Jammula, S., Kota, S.K., Krishna, S.V., Meher, L.K., *et al*. (2013) Endocrinology of Parturition. *Indian Journal of Endocrinology and Metabolism*, **17**, 50-59. https://doi.org/10.4103/2230-8210.107841

[16] Slack, E., Best, K.E. and Heslehurst, N. (2019) Maternal Obesity Classes, Preterm and Post-Term Birth. *BMC Pregnancy and Childbirth*, **19**, Article No. 434. https://doi.org/10.1186/s12884-019-2585-z

[17] Kaze, F.F., Njukeng, F.A., Kengne, A.-P., Ashuntantang, G., Mbu, R., Halle, M.P., et al. (2014) Postpartum Trend in Blood Pressure Levels. *BMC Pregnancy and Childbirth*, **14**, Article No. 134. https://doi.org/10.1186/1471-2393-14-134 http://www.biomedcentral.com/1471-2393/14/134

[18] Samuel, E.J. (2014) Auto Immunity and Fragmented Germ Cells. *IOSR*: *Journal of Medical and Dental Sciences* (*IOSR-JDMS*), **13**, 99-105.

[19] Samuel, E.J. (2014) Abortion, Contraception Reversal-Medical Miracle-Autologous Germ Cells Replant-Decline in Global Degenerative, Autoimmune, Neoplastic, Infectious Diseases. *IOSR Journal of Dental and Medical Sciences*, **13**, 93-102.

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