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A Case Report of Monomorphic Epithelial Intestinal T-Cell Lymphoma and Literature Review

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Abstract

Background: Monomorphic epithelial intestinal T-cell lymphoma (MEITL), previously known as type II Enteropathy-associated T-cell lymphoma (EATL), is a rare intestinal tumor with strong invasiveness, poor prognosis, atypical clinical manifestations, and is difficult to diagnose. **Aim:** The clinical manifestations, imaging, histopathology, and immunohistochemical characteristics, treatment and prognosis of this case of MEITL were analyzed retrospectively, and combined with literatures learning to provide the experiences and lessons for diagnosis and treatment. **Case Presentation:** Here, we present a 68-year-old Asian woman with unexplained intestinal perforation and lung imaging changes as the first manifestation, and gradually appearing refractory diarrhea and pulmonary cavities. During the period, due to the unknown diagnosis, all treatments were mainly based on anti-infection and antidiarrhea, but her symptoms had no improvement after therapy, even died of poor basic physical conditions. Colonoscopy biopsy showed atypical lymphocyte infiltration-like growth and the formation of local superficial ulcers. Immunohistochemical tests showed that T-lymphocyte hyperplasia was predominant and led to a diagnosis of MEITL. **Conclusion:** MEITL is mainly manifested by gastrointestinal symptoms and lacks specific symptoms. The diagnosis of MEITL should be based on comprehensive judgment of clinical manifestations, pathological features and immunohistochemical detection results. Positive biopsy confirmed and timely chemotherapy may be conductive to a better prognosis.

Keywords

MEITL, Pulmonary Cavities, Refractory Diarrhea, Intestinal Perforation

*Corresponding author.

1. Introduction

EATL is a rare intestinal intraepithelial T-cell lymphoma, which is a subtype of non-Hodgkin's lymphoma. It consists of two types, Type I (classic) EATL, composed of large tumor cells and typically manifested as celiac disease, accounts for 80% - 90% of all cases, which were mainly distributed in European countries. The other type of EATL, composed of small to medium-sized tumor cells, was mainly distributed in Asian countries, which accounted for 10% - 20% [1]. In the newly revised WHO lymphoma typing of 2016 [2], type II EATL was renamed as MEITL. Some international reports pointed out that affected parts of MEITL are mainly involved in the abdominal cavity and intra-abdominal lymph nodes with gastrointestinal symptoms such as diarrhea as the main manifestation, occasionally found in the skin, ovary, chest and other extraintestinal organs; however there were no reports of combined lung changes as the initial performance. Here, a special MEITL case is presented with the permission of the patient, the clinical manifestations, pathological features, diagnosis and treatment process was summarized and discussed.

2. Case Report

A 68-year-old woman was diagnosed as "small intestine perforation" due to "abdominal pain" on May 20, 2018. Then emergency surgery was performed, but the reason of perforation was not found out during the operation. Then the chest CT showed "pulmonary infection with pleural effusion" during hospitalization, but no signs of infection. The symptoms were improved by giving anti-infective treatment and the patient was discharged. After re-examination, pleural effusion disappeared, but "pulmonary infection lesions" still existed and gradually expanded to form multiple cavities. The patient was hospitalized again because of coughing for half a year, exacerbating for three months on August 8, 2018. During that period, two bronchoscopy biopsy showed inflammatory changes and one ultrasound fiber optic plexus biopsy showed multiple lymphocytes. The result of PET/CT examination combined with medical history was considered to be neoplastic lesions, but no histopathological evidence, except anti-tumor treatment, other treatments were performed for the patient such as anti-infective (Levofloxacin, Piperacillin tazobactam), relieving cough and reducing sputum, but the symptoms were not obviously improved and sputum with blood streaks was occasionally found, then the magnetic navigation puncture and surgery were recommended to diagnosed, but the relatives of patient refused, and the conservative treatment was discharged after more than 20 days. During the hospitalization, the patient has diarrhea 2 - 3 times a day, and montmorillonite powder was given for treatment. After discharge, the diarrhea gradually increased to more than 10 times a day with yellow bloating, accompanied by abdominal pain, poor appetite, vomiting, and weight loss. Then the patient came back to hospital again on September 25, 2018.

After hospitalization, chest CT (**Figure 1(a)**, **Figure 1(b)**) showed multiple

cavities, nodules and solid changes in the lungs. These symptoms were recommended to identify chronic infection and tumor metastasis. Abdominal CT enhancement (**Figure 1(c)**, **Figure 1(d)**) showed rectal and sigmoid edema, which could be potentially inflammatory. Colonoscopy (**Figure 2(a)**, **Figure 2(b)**) showed irregular shallow ulcers with different sizes were distributed in the end of ileum, ascending colon, descending colon, and sigmoid colon, respectively. Pathology (**Figure 3(a)**): the infiltrating growth of lymphoid cells with small volume in the mucosal epithelium was observed and infiltrated into the lamina propria. Morphology and immunohistochemistry were consistent with monomorphic epithelial intestinal T-cell lymphoma (MEITL). *In situ* hybridization of tumor cells: EBER (−). Immunohistochemistry shows (**Figures 3(b)-(f)**): LCA (+), CD3 (+), CD2 (+), CD7 (+), CD43 (+), CD5 (−), CD56 (+), Perforin (−), Granzyme B (−), CD20 (−), CD79a (−), CD4 (−), CD8 (−), CD138 (−), CD30 (−), CD34 (−), VIM (+), pCK (−), Ki -67 (LI: 50%).

Laboratory examination results: the patient had no obvious blood abnormalities in the blood routine in the past six months, but mild anemia, malnutrition and electrolyte imbalance occurred in the late stage due to long-term intractable diarrhea. Liver and kidney function, coagulation function, rotavirus detection, thyroid function, a complete set of lupus, pANCA, cANCA, PPD test, stool culture, IgA, IgG, IgM, complement C3, RF, anti-CCP antibody, ASO, T-SPOT and clostridium difficile culture of stool were normal. Complement C4 0.38 g/L ↑;

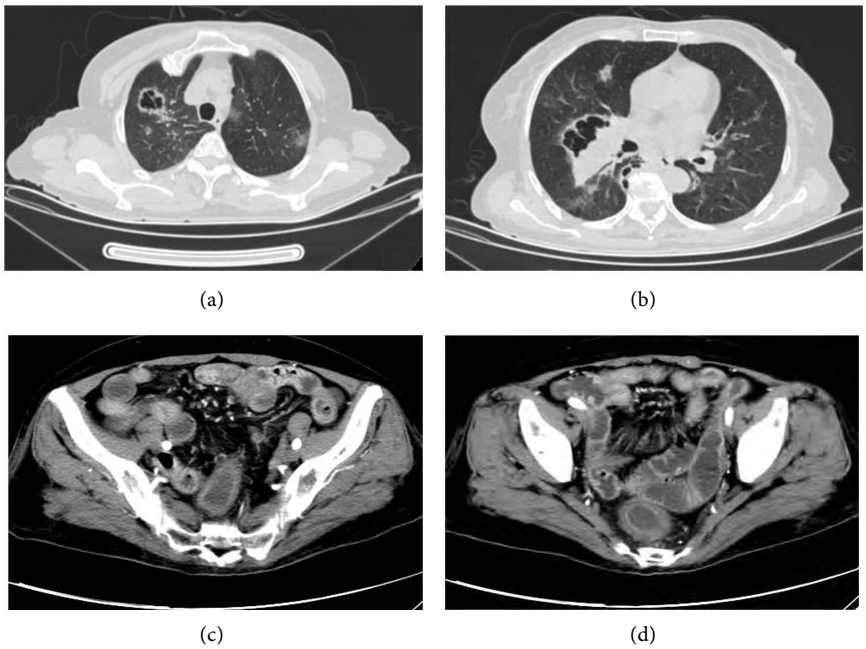
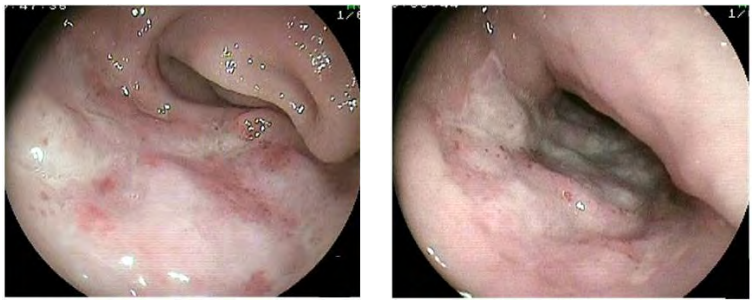
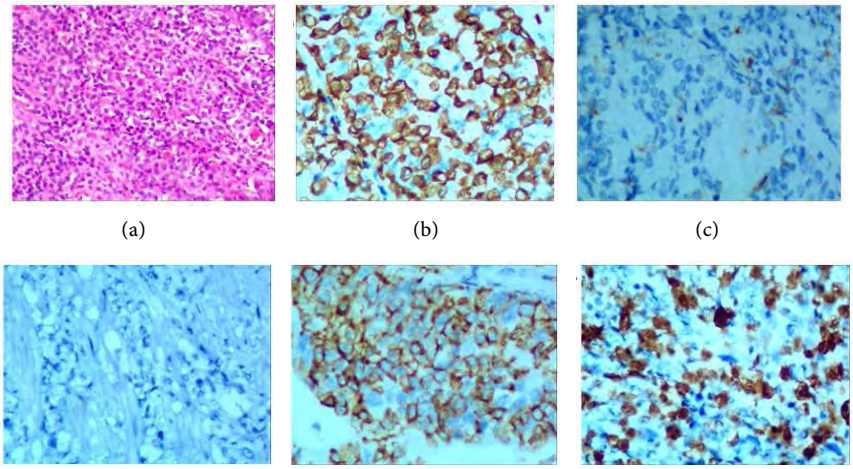


Figure 1. (a) (b) CT scan of the chest in multiple sites showed multiple cavities in the lungs and exhibited a honeycomb change. Multiple nodules were obvious in the lungs and some of them exhibited glass-like changes; (c) (d) Abdominal CT enhancement showed that thickness of the rectum and sigmoid colon wall increased, enhanced mucosal was mildly intensified and exhibited concentric ring-like changes, peripheral fat gap became blurred.



(a) (b)

Figure 2. Multiple irregular shallow ulcers can be observed in many parts of the large intestine under colonoscopy with clear borders, covered with white moss, soft texture. The granulation tissue changes at the bottom of the sigmoid colon.



(a) (b) (c)

(d) (e) (f)

Figure 3. Immunohistochemistry observations of MEITL tissue ((a): HE*200); Tumor cells showed positive for CD3 (b), negative for CD8 (c), CD30 (d), CD56 (e), Ki-67 index of 50% (f).

C-12 tumor marker: CA199 37.27 IU/mL ↑; CA125 35.63 IU/mL ↑; Cyfra21-1 8.70 ng/ml ↑; PCT 0.26 ng/ml; ESR 21.00 mm/h ↑, CRP 55.70 mg/L ↑. Sputum culture: *Enterobacter aerogenes*.

During the hospitalization, the patients were given symptomatic treatments such as antidiarrheal (Montmorillonite), anti-infection (Cefoperazone), stomach protection (Pantoprazole) and nutritional rehydration, but the condition was getting worse, even with a dramatic loss of weight. Because of poor effect of chemotherapy, extremely easy access to intestinal perforation and poor prognosis, the patient and his families refused to chemotherapy and discharged. One month later, the patient died.

3. Discussion

It is difficult to diagnose MEITL because of the extremely low morbidity, lacking of characteristic symptoms and endoscopic features. The case we presented here is combined lung changes and intestine perforation as the initial performance,

without considering these two symptoms as a manifestation of one disease, which caused misdiagnosis and missed diagnosis. It's finally confirmed until half-year data of the patient was collected for consultation and the pathological specimens with special staining were sent to the pathology department of the superior hospital.

3.1. Clinical Manifestation

MEITL is mainly found in middle-aged and elderly men, with a male to female ratio of about 6:1, mainly involved in the abdominal cavity and intra-abdominal lymph nodes with gastrointestinal symptoms such as diarrhea as the main manifestation, more common in the small intestine, followed by the large intestine and stomach. In combination with previous reports of such cases at home and abroad, the disease has various symptoms such as skin damage caused by skin infiltration [3], ascites, ovarian mass [4], pleural effusion [5], central nervous system [6], autoimmune hemolytic anemia [7], etc. Other non-specific manifestations include abdominal pain, diarrhea, abdominal mass, constipation, etc. systemic manifestations of anorexia, weight loss, etc. which was often misdiagnosed as infectious enteritis, inflammatory bowel disease and intestinal tuberculosis that can explain the patient's chronic diarrhea with obvious symptoms of consumption [8] [9]. Sometimes, intestinal perforation and obstruction are the first manifestations, thus we should pay attention to identify such common diseases. In this case, pulmonary cavity changes are considered to be the most likely metastasis of MEITL in the lungs, with metastasis in the early stage. However, due to the low incidence of this disease, three fiberoptic bronchoscopic biopsies did not perform special staining, so no diagnostic results were found, but combined with imaging findings, pulmonary cavities manifested in immunocompromised patients may result from bacterial infection, aspergillosis, tuberculosis or non-tuberculous mycobacterial etc. [10]. So it's not ruled out that this case is MEITL combined with other diseases. The disease progressed rapidly and the prognosis was poor, the onset to the final diagnosis was 5 months and the survival period was 6 months while the median survival time was only 11 months in previous reported [11]. The main causes of death of this disease are malnutrition, infection, systemic failure, etc.

3.2. Pathology and Endoscopic Features

The diagnosis of the disease mainly relies on pathological examination. Some patients are diagnosed due to "acute abdomen" undergoing surgical resection of the intestinal segment biopsy, other patients depend on endoscopic biopsy. Commonly used endoscopic biopsies include enteroscopy, gastroscopy, and colonoscopy. A small portion of cases makes a definite diagnosis by a biopsy of extraintestinal organs and tissues. Enteroscopy is an expensive and invasive procedure, and difficult to operate, which is not the preferred endoscopy for disease diagnosis. In the early endoscope stage of MEITL, the main manifestations are ulcers of different depths, with no special forms, which caused most of

them were misdiagnosed as Crohn's disease or intestinal tuberculosis. The middle and late ulcers deteriorate into penetration and develop into large and deep ulcers, where the surface is uneven, the bottom is covered with white moss and around the ulcer as insect eroding, normal mucosa could be observed between the ulcers, and intestinal tube with ulcers are obviously edema and thickened, but generally no intestinal stenos is [12]. The pathogenesis of MEITL may be related to glucan enteropathy and EB virus infection but the specific mechanism is unknown [13]. In this case, EBER (−), but human anti-glutenin antibodies (AGA) could not be determined due to lack of technology. MEITL is a rare type of lymphoma that originates from intestinal T lymphocyte. Immunohistochemically, tumor cells are CD3+, CD5−, CD4−, CD8+, CD56+, CD30−, and EBER− [14].

3.3. Treatment

There is currently no standardized treatment. Some scholars believe that surgery can remove lesions, reduce tumor burden, avoid bleeding and perforation, but the rate of curative resection is low and postoperative complications are common. Another part of the reports suggests that surgery combined with chemotherapy or chemotherapy alone would be the first choice. The most commonly used chemotherapy for MEITL in previous reports of domestic and foreign is anthracyclines and CHOP [15] or R-CHOP (rituximab, cyclophosphamide, doxorubicin, vincristine, prednisone) [14]. A few reports have tried other therapy such as gemcitabine instead of anthracyclines [14], hyper-CVAD (cyclophosphamide + doxorubicin + vincristine + dexamethasone/methotrexate + cytarabine) [4] and Polyethylene glycol combined with asparaginase [16], etc. Some patients have a survival period that exceeds the expected median survival, but the efficacy remains to be confirmed by more cases. In this case, the patient is mainly treated symptomatically, chemotherapy is recommended after pathological diagnosis, but treatment is abandoned because of poor prognosis. Currently all treatments for MEITL patients show low survival rate, which require more research to find out innovative therapies, such as transplantation, targeted drug and autologous stem cell transplantation after high-dose chemotherapy may be a better option for MEITL treatment [17].

4. Conclusion

In summary, because of the low incidence and difficulty to distinguish from Crohn disease, intestinal tuberculosis and other types of lymphoma of the gastrointestinal tract, MEITL is hard to diagnose and treat in time. It invaded and progressed rapidly, with poor response to current treatment, resulting in a bad prognosis. During the diagnosis, it should be highly suspected to be MEITL if the patient has unexplained refractory diarrhea, pulmonary cavities, intestinal perforation, no efficacy after long-term conservative treatment and is combined with multiple ulcers of the intestine and systemic symptoms. Then active biopsy should be combined with immunohistochemistry, and the results are recom-

mended to obtain a comprehensive judgment to avoid missed diagnosis or misdiagnosis.

Conflicts of Interest

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

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Hashimoto Thyroiditis: Simple Diagnosis with 2D-Shear Wave Elastography

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Abstract

Background: Hashimoto thyroiditis diagnosis is primarily established on clinical and laboratory findings; however, some hashimoto thyroiditis cases are euthyroid and seronegative. Moreover, these patients might also have normal conventional ultrasound findings. **Aims:** In our study, we aimed to distinguish the typical background characteristics of hashimoto thyroiditis using virtual touch tissue imaging quantification maps obtained with acoustic radiation force impulse imaging. **Methods:** Our study consisted of 28 hashimoto thyroiditis patients without characteristics of ultrasound findings and 28 healthy subjects. The thyroid parenchymal tissue mechanical properties were analyzed with the virtual touch tissue imaging quantification after ultrasound examination, and then related colored maps were obtained. Shear wave velocities were recorded (m/s) from the homogenous area where hardest and softest points were closest to each other on virtual touch tissue imaging quantification maps. The difference between the minimum and maximum shear wave velocities for each case (Δv) was calculated and recorded. **Results:** Assessment of virtual touch tissue imaging quantification maps revealed a significant difference between hashimoto thyroiditis and control groups in terms of maximum SWV's, but no significant difference was observed between the minimum shear wave velocities ($p < 0.05$). Notably, a significant difference was observed when only Δv values were taken into account. In summary, the effects of chronic autoimmune thyroiditis can be dis-

tinguished by simply using Δv on virtual touch tissue imaging quantification maps. When the cut-off value of Δv was accepted 0.42, the diagnosis of hashimoto thyroiditis could be made with 88% accuracy. **Conclusion:** We suggest that shear wave velocities measurement on virtual touch tissue imaging quantification maps is a promising method in equivocal hashimoto thyroiditis cases, in which the diagnosis of hashimoto thyroiditis is unachievable with clinical, laboratory and conventional ultrasound findings.

Keywords

ARFI, Hashimoto Thyroiditis, SWE, SWV, VTIQ

1. Introduction

Hashimoto's thyroiditis (HT) is an autoimmune disease that eventually leads to thyroid gland dysfunction. HT has a relatively high prevalence, and predominantly affects women [1] [2] [3]. HT diagnosis established on clinical and laboratory findings; however, 5% and 13% of all HT cases are euthyroid and seronegative respectively while 10% of the female population have positive anti-TPO and Anti-Tg serology without HT. Furthermore, thyroidectomy specimens of some cases, whose clinical and laboratory findings are incompatible with HT, were diagnosed as HT after the histopathological analysis [4] [5] [6]. These findings suggested that the laboratory and clinical findings alone might not be enough for the diagnosis in some cases. However, despite inconsistencies of the clinical and laboratory aspects aforementioned above, the clinical findings and blood biochemical analyses generally were considered as adequate for the diagnosis of the disease, and additional thyroid ultrasound (US) examination was not commonly requested for the HT diagnosis. On the other hand, HT cases are mostly followed up with the periodic US owing to the elevated risk of papillary thyroid neoplasms. Nevertheless, despite not routinely requested, US examination might be a problem-solving tool in some patients with equivocal clinical and laboratory findings. However, some of the euthyroid and seronegative HT patients might not have noticeable characteristic parenchymal changes of HT disease on US. Moreover, conventional US findings are qualitative, subjective and operator dependent.

Recent studies claimed that shear wave elastography (SWE), a novel elastographic method that quantifies the elasticity of structures by tracking shear waves passing through them, aids to recognize and discriminate the parenchymal pattern in HT [7] [8].

In this study, we aimed to test whether it is possible to recognize background parenchymal changes occurring in thyroid gland in equivocal HT, whose grayscale findings are not characteristic for HT, using virtual touch tissue imaging quantification (VTIQ) maps obtained with acoustic radiation force impulse imaging.

2. Materials and Methods

This prospective study was conducted between January 2014 and January 2015. The study was approved by the local ethics committee, and written consent was obtained from all the patients. The patients with a normal blood level of thyrotropin receptor antibodies (TRAbss < 9.0 U/L), high levels of anti-TPO (thyroid peroxidase antibodies, 0 - 60 IU/ml) and anti-Tg (thyroglobulin antibodies, 0 - 60 IU/ml) were exclusively selected for the patient group. All patients had to have normal thyroid function tests (TSH: 0.51 - 4.94 uIU/ml, fT4: 10.7 - 18.4 pmol/l, fT3: 3.93 - 7.70 pmol/l) to be included in the study since our aim was to evaluate HT patients with obscure clinical findings. Therefore, diagnosis of HT relied on thyroid autoantibodies and clinical findings. All patients were questioned and investigated particularly to exclude any other disease or condition, which might elevate anti-TPO and anti-Tg autoantibodies to ensure elevated levels of autoantibodies were secondary to HT rather than any other systemic or local disease. A single radiologist evaluated all the patients (D.Y with 20 years of ultrasound and 9 years of elastography experience) using Siemens S2000 Helix (US-Siemens Medical Solutions, Erlangen, Germany) with 9-Hz linear transducer. All examinations were performed from the right lobe of the thyroid gland in the axial plane. A detailed grayscale US examination was performed before SWE measurement. All of the patients had to have normal thyroid volumes but not to have parenchymal changes specific to HT disease, prominent heterogeneity and pseudonodular appearance, which might substantially suggest HT [9] [10] [11]. Patients with abnormal thyroid volume and parenchymal changes on US were excluded from the study. Patients were asked to hold their breath for 5 seconds after normal inspiration for SWV imaging. The operator placed transducer providing gentle skin contact without applying compression. VTIQ color maps were obtained in which the blue color shows soft, and the red color shows hard tissue. On VTIQ maps, shear wave velocity (SWV) was recorded (m/s) from a homogenous area where the hardest and softest points were closest to each other at the same depth. The observer captured five separate VTIQ color maps, and then chose the most appropriate one to perform SWV measurements. The maximum SWV value, measured from the hardest point, and minimum SWV value, measured from the softest point, and the difference between the minimum and maximum speed rate for each case (Δv) were calculated. **Figure 1** demonstrates SWV measurements on a SWE colored map.

Statistical Analyses

Independent Student t-Test or Mann Whitney U Test was used for testing the difference between groups for independent samples depending on normal distributed. The normal distribution of the data was tested by Shapiro-Wilk test. Receiver operating characteristic (ROC) curve analysis was carried out to calculate 95% confidence intervals of the area under the curve (AUC) to identify the best cut off points of difference between the minimum and maximum SWV

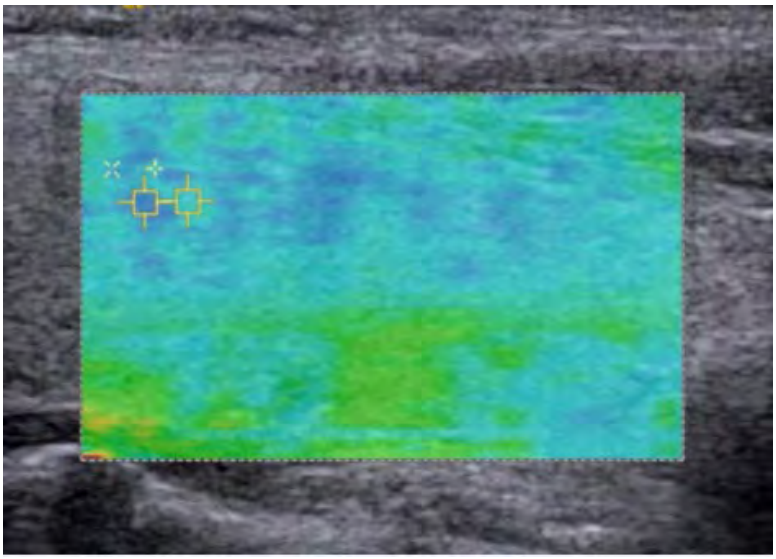

Figure 1. A 35-year-old female patient with HT. The SWV measurement is performed from the homogenous area where hardest and softest points were closest to each other at the same depth. x: softest point, 1.79 m/s; +: hardest point, 2.25 m/s. Δv is calculated as 0.46, which is higher than the cut-off value (0.42 m/s), suggesting HT.

(Δv). The highest value for Youden's index was accepted as the best cutoff value. Youden's index was obtained from coordinates of the curve and calculated by "J = max [SN + SP] − 1". The sensitivity and specificity of the cutoff values were also presented. All data analysis was performed with SPSS 19 for windows and was reported with 95% confidence intervals, $p < 0.05$ was considered significant.

3. Results

Finally, 28 patients (27 female, 1 male, mean age: 37), and 28 healthy participants (27 female, 1 male, mean age: 39) were included in the study. The mean age of the patients was 33.4 ± 5.2 years and the mean age of the healthy controls was 32.3 ± 4.8 years. There was no significant difference in demographic characteristics between the study and control groups. There was a significant difference the mean maximum SWV values of the patients (was 2.36 ± 0.22 m/s), and the control group (2.08 ± 0.34 m/s) ($P = 0.001$). The mean minimum velocity values were not statically differs between the patient (1.83 ± 0.20 m/s) and the control group (1.84 ± 0.30 m/s) ($P = 0.884 > 0.05$) (Table 1).

Moreover, when Δv value is taken into account, there is a high significance difference among the patients (0.53 ± 0.14) and the control groups (0.24 ± 0.12) ($P < 0.001$) (Table 2).

A receiver operative characteristic (ROC) analysis was applied for the cut-off value of the velocity difference, which is a distinctive feature of the study and control groups. Area Under the Curve (AUC) value was remarkably high ($P < 0.001$, AUC = 0.931, 95% CI: 0.87 - 0.99). Accordingly, when the cut-off value as set to 0.42, the parenchymal ground influenced by HT could be detected with 79% sensitivity, 96% specificity, and 88% accuracy rate (Table 3, Figure 2).

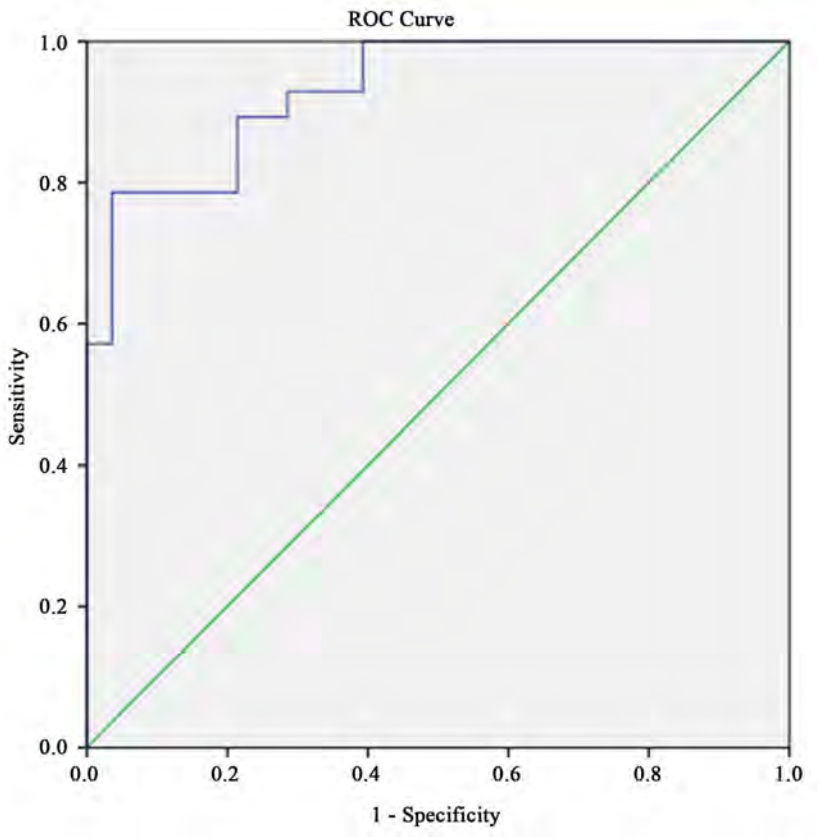

Figure 2. The receiver operative characteristic (ROC) analysis, 79% sensitivity, 96% specificity, and 88% accuracy rate.

Table 1. SWV max and min values in the study and the control groups.

		n	Mean ± S.D.	Min.-Max.	Median	p
Max	**Control**	28	2.08 ± 0.34	1.42-2.66	2.02	0.001[a*]
	Study	28	2.36 ± 0.22	1.90-2.88	2.34	
Min	**Control**	28	1.84 ± 0.30	1.22-2.35	1.88	0.884[a]
	Study	28	1.83 ± 0.20	1.44-.31	1.84	

a.Independent samples test, *significant.

Table 2. SWV difference in the study and the control groups.

		n	Mean ± S.D.	Min. - Max.	Median	p
SWV difference (Δv)	**Control**	28	0.24 ± 0.12	0.04 - 0.49	0.21	<0.001[a*]
	Study	28	0.53 ± 0.14	0.26 - 0.74	0.55	

a.Independent samples test, *significant.

Table 3. ROC analysis with related **Figure 2**.

	Cut-off	SN. (%)	SP. (%)	Accuracy (%)	AUC (%)	p
SWV difference (Δv)	0.42	79%	96%	88%	0.931	<0.001[a*]

a.ROC curve, *significant.

4. Discussion

Grayscale ultrasound findings like heterogeneous echo-structure with pseudo nodules, and Doppler US findings such as increased or hierarchical vascularity are distinct features for HT; nonetheless, these modalities have low diagnostic value owing to their subjective characteristics, and also their various presenting degrees in different cases. Even though several ultrasound texture analysis studies aimed to overcome these problems, since the post-processing is relatively sophisticated and nonspecific, all suggested techniques gained no acceptance for routine daily ultrasonography practice. Furthermore, some studies pointed out that texture analyses have no superiority over conventional grayscale US assessment in the differentiation of HT [12] [13].

SWE is mainly used to differentiate benign and malignant thyroid nodules, but it could also be used in the diagnosis of HT. There are a few numbers of studies available about SWE in the literature, which primarily aimed to differentiate benign and malignant nodules in HT patients [14] [15] [16] [17]. Some other SWE studies sought to discover the probable background elasticity changes developed by chronic autoimmune thyroiditis [14] [18] [19] [20].

The SWV values that are acquired with different hardware and/or software may affect the results. Fukuhara *et al.* and Hekimoglu *et al.* conducted two separate studies, and concluded that thyroid glands of the cases with chronic autoimmune thyroiditis (CAT) have relatively higher SWV values. They observed SWV values that reach up to 2.56 ± 0.57 m/s and 2.56 ± 0.30 m/s in the patients with CAT [19] [20]. On the other hand, Magri's study in which the measurement obtained and recorded in kilopascal unit suggested that there was no statistically significant difference between parenchymal ground SWV values of normal and HT cases [14].

Senile or sequela (post-treatment) changes may also have a heterogeneous hypo-echogenic parenchymal pattern; thus, mimic the appearance of HT background. More information could be gathered about the disease when these parenchymal patterns are correctly assessed and differentiated from the HT [21].

There are some reported cases with postoperative histopathological definite HT diagnosis, in which clinical and laboratory data were not compatible with HT [4] [5] [6]. In light of all the aforementioned issues, it is vital to diagnose HT at an early stage, especially in the areas where the disease is endemic.

Our study differs from the others by investigating Δv value, which we suggest that it could be accounted as a more reliable method to detect parenchymal background changes in HT. We highlight that both normal patients' thyroid parenchyma and HT patients' thyroid parenchyma might demonstrate soft and hard areas on VTIQ color map, which might arise some challenges to the operators, especially inexperienced ones, in deciding to choose the hardest or softest area on VTIQ map. Unfortunately, inappropriate detection of the hardest or softest area might yields improper measurements might yield false negative or positive results on SWE examination. On the other hand, given the heterogeneity of the gland in HT disease, SWV differences within the gland are substantially

more prominent than the normal gland. Thus, if even the operator does not visually able to determine softest or hardest area, calculating the difference, namely Δv value, the value between the softest and hardest area that visually and subjectively identified by the observer, might be more accurate owing to prominent stiffness differences within gland due to parenchymal background changes of HT.

Nevertheless, we acknowledge that there are several limitations in our study. First, our study is the absence of Graves' disease, which is the most important group of diseases that can mimic HT. Hence, our method should be tested on larger series in the centers with frequent cases of Graves' and HT. Second, we did not be able to do histological confirmation, but we had no chance but to rely on the diagnosis we achieved with clinical-laboratory thyroid function indicators and autoantibodies since thyroid biopsy or surgery is not necessarily indicated to diagnose HT in our country. Third, we had a small number of participants since we strictly formed our inclusion criteria as we mentioned in the materials and methods section. Fourth, we did not test the reproducibility of the SWV measurements; however, many studies have tested and demonstrated both inter and intraobserver correlation of the SWV measurements of the thyroid gland is considerably high with relatively straightforward learning curve [22]. In this study, our cases were evaluated without separation of HT into acute exacerbation period or subacute-chronic period. SWV and Δv values may be measured in larger patient populations divided into these groups according to clinical, laboratory and sonographic characteristics. Finally, we did not evaluate the relationship between the SWV values and the laboratory values of the patients since the main scope of our study was to simply offer an auxiliary method in the diagnosis of HT. The correlation between the SWV measurements and the laboratory results of the patients should be assessed in the future studies with a larger population.

5. Conclusion

In conclusion, with the contribution of SWE, the velocity difference (Δv) between the hardest and the softest areas on thyroid gland parenchyma could be determined through SWV colored maps (VTIQ); thus, it would be straightforward to define whether the changes on the ground are secondary to HT or not. When the cut off value for Δv is taken as 0.42 (m/s), background influenced by HT can be detected with 96% sensitivity and 88% accuracy by this method alone. Even the other parameters are not available to utilize or impractical to use, Δv may provide valuable information in the one-stop diagnosis of the HT. However, further studies that would test our findings in larger series with other diffuse parenchymal diseases, especially Graves' disease, are needed to reveal the actual value and availability of this method.

Conflicts of Interest

All authors declare no conflict of interest.

Ethical Statements

All procedures followed were in accordance with the ethical standards of the responsible committee on human experimentation (institutional and national) and with the Helsinki Declaration of 1964 and later versions.

Informed consent was obtained from all patients for being included in the study.

Financial Disclosure

The authors have no relevant financial interest in this article.

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A Prognostic Model of the Development of Postpartum Purulent-Inflammatory Diseases

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Abstract

Background: Currently, postpartum purulent-inflammatory diseases continue to be a prominent issue in medicine. As a result, numerous scientific publications were devoted to finding the solution to this issue. Primarily these solutions included the idea of optimisation of antibiotic-based disease prevention and therapies. However, the early diagnosis and prognosis of these pathologies were unfortunately overlooked. **The Aim of the Study:** To build a prognostic model of the development of postpartum purulent-inflammatory diseases. **Material and Methods:** The main focus of our research was establishment of methods of early diagnosis and prognosis of purulent-inflammatory diseases. The main cohort consisted of 170 women diagnosed with purulent-inflammatory diseases while the control cohort was made of 40 women with an uncomplicated course of pregnancy; patient's blood serum was analysed using fluorescence spectroscopy. Additionally, we implied a variety of standardised algorithms used during clinical and laboratory examination of the patients with postpartum endometritis. **Results:** Fluorescence spectra were studied for 40 women of control group and 170 women of the main group. Based on the data obtained using fluorescence spectroscopy and data from clinical and laboratory examinations (extragenital pathology, gynecology-related diseases, risk of miscarriage, surgery, TORCH-infections, colpitis, labour duration > 12 hrs, labour anomalies, maximum blood serum fluorescence spectrum values, fluorescence spectrum ≤ 0.845, age, number of bed days, fetal distress), we have derived a prognostic model of the development of postpartum purulent-inflammatory diseases. **Conclusion:** As a result, we derived a prognostic model based on the main 13 factors, which contribute to

development of postpartum purulent-inflammatory diseases. This model was determined correct with a probability of over 99% ($p < 0.001$; $\chi^2 = 174.74$; df = 13).

Keywords

Postpartum Purulent-Inflammatory Diseases, Prognostic Model, The Method of Logistic Regression, ROC-Analysis

1. Introduction

Currently, one of the cornerstone issues in modern obstetrics is postpartum purulent-inflammatory diseases (PPPID). Over the past decade, the frequency of PPPID remained between 5% - 20% and despite availability of abundance of medical treatments, it remained one of the core factors behind maternal mortality [1]. Therefore, it will be fair to conclude that this is a crucial issue which has not been resolved as of yet.

Numerous publications were dedicated to this topic, as such they were mostly highlighting optimisation of the existing therapies involving antibiotics. However unfortunately, evaluation of the prognosis and early diagnosis of development of PPPID were severely neglected [2].

A study of individual traits of the patient's organism, in particular, analysis of genetic predisposition to appearance of obstetric and perinatal complications is all a part of predictive medicine. Predictive medicine enables preventative action towards aforementioned complications due to involvement of both prophylactics and determination of aetiological factors and pathogenic mechanisms [3].

High sensitivity and accuracy are the key factors why physical methods of investigation are viewed as highly effective in medical practice. Therefore, the data obtained from clinical and laboratory analysis are core for prognosis of PPPID.

2. Literature Review

It is important to emphasize that the most available diagnostic method for inflammatory diseases is a blood serum test. However, it is also crucial to highlight the findings of other researchers [4] that a diagnostic role and accuracy of leukocyte indicators and leucocytosis itself have not been proved effective in predicting postpartum infections. This finding made us question significance of leucocytosis in administration of antibacterial therapy, because we failed to establish a statistical significance between bacterial infection and leucocytosis overall.

Subsequently, C-reactive protein (CRP) and procalcitonin (PCT) are the most investigated, known and used markers of inflammatory process [5] [6] [7]. CRP is considered a "gold standard" in diagnostics of human inflammatory processes. Moreover, the presence of CRP in blood serum has been implied as an inflam-

matory process marker during pregnancy, preterm premature rupture of membranes and postpartum complications.

An increase in PCT is observed during systemic inflammation during either complex bacterial infections or sepsis. It is important to highlight that PCT reaches its peak values much earlier than CRP. However there is also an opinion that variation in PCT values is not necessarily indicative of an infection or the course of an inflammatory process, especially when there is a presence of haemodynamic instability [7].

Heavy bacterial infections and sepsis contribute to an elevation in PCT concentration in blood serum as a result of extrathyroidal synthesis, taking place in leukocytes, neuroendocrine lung cells and in liver (influenced by pro-inflammatory stimulators). PCT (endotoxins) and pro-inflammatory cytokines IL-6 and TNF are classed as core synthesis inductors. A combination of procalcitonin and the rest of clinical and physical data are considered to be diagnostic markers of sepsis, inflammatory reactions and severity of sepsis among complex patients.

Electron paramagnetic resonance (EPR) analysis of detoxifying efficiency [DTE] of albumin was proposed by another group of researchers as an option for forecasting postpartum endometritis [8] [9].

Logistic regression was implied as a method of statistical processing of the results and data obtained from the experiment. We established the correlation, where DTE ≤ 40% means a high risk of postpartum endometritis, while when DTE ≥ 70% the risk of postpartum endometritis is absent.

A lot of clinical, laboratory and instrumental data is collected during treatment, and there are two core tasks where it is crucially required. First of all, it is development of diagnostics for the disease, especially in preclinical stage. Secondly, prognosis of appearance and development of the aforementioned disease.

During our investigation, along the conventional means of diagnostics, we also implied fluorescent spectroscopy [10] (patent of Ukraine N˚ 33472) as a way to optimize treatment of patients with PPPID. Previously, the aforementioned method was successfully involved in sepsis diagnosis [11] (the patent of Ukraine N˚ 76953) and purulent and sepsis-related complications in gynaecology and obstetrics [2] [12] [13].

Blood serum stimulation was done under wavelength of 280 nm, which corresponds to the range where human albumin is in its' excited state. Although, it is important to point out that there are conformational changes in albumin molecule in blood serum as a result of progression of purulent-inflammatory diseases due to endogenous intoxication; this is related to interaction between albumin molecules and bacterial products of metabolism. During our investigation we were able to determine that a structurally altered albumin would lead to alterations in blood serum fluorescence. Due to the fact that aforementioned method allowed us to determine changes in blood serum within a timespan between 24 - 48 hours prior to any clinical signs, we can confirm that it be suc-

cessfully implied in sepsis diagnosis [2] [11] and monitoring.

We carried out an evaluation of the confirmed risk factors of postpartum endometritis development based on the results from both fluorescent spectroscopy, clinical and laboratory data [14]. Currently, there is a number of approaches used in terms of prognostic models. Logistic regression is considered to be one of the more common approaches [15] [16]. However, these scientists have also argued that there are few complications in implementing it in hospitals due to a lack of training among medical professionals.

Aim: to build a prognostic model of development of postpartum purulent-inflammatory diseases.

3. Data and Methodology

The clinical research centre for this particular investigation was the Department of Gynecology N˚ 2 of Vinnytsia Council Clinical Hospital N˚ 2. The luminescent laboratory of the Department of Experimental Physics, Ivan Franko Lviv National University was an experimental research centre. The research study took place between 2014 and 2018 inclusively.

The main cohort of patients consisted of 170 new mothers with postpartum endometritis. Women with single pregnancy, who had histologically confirmed diagnosis of PPPID in postpartum period were involved in the research. These women were informed beforehand and gave an informed consent for participation in the aforementioned study. Postpartum period after multiple pregnancies, period after antenatal death of the fetus, decompensated somatic illnesses, presence of primary immunodeficiency among postpartum women, presence of HIV infection, tuberculosis (pulmonary and extra-pulmonary), diabetes and a presence of oncological pathology were all considered as exclusion criteria. The control group was made of 40 new mothers with uncomplicated course of the postpartum period. The age of the patients of main cohort and control group was from 18 to 40 years. They were all European and lived in the Vinnytsia region of Ukraine.

Methods of investigation: clinical, laboratory, biochemical, instrumental (uterus and ovarian sonography, bacteriologic and histologic analysis of metroaspirate, fluorescent spectroscopy), mathematical and statistical (logistic regression and ROC-analysis).

3.1. Data Source

As a part of our investigation we thoroughly analysed 40 factors which characterised the unique features of the course of pregnancy, postpartum period and the clinical data obtained from both main and control cohorts of patients. We carried out a detailed analysis of the lab examinations (general blood and urine tests, biochemical blood tests, immunofixation analysis for TORCH-infections, bacterioscopy of vaginal and cervical samples), results of instrumental examination (ultrasonography of lesser pelvis, histological analysis of metroaspirate, fluorescent spectroscopy) and statistical methods (logistic regression and

ROC-analysis).

3.2. Research Results

During this investigation we analysed main indicators of 170 new mothers with PPPID (main cohort) and 40 new mothers with uncomplicated course of postpartum period (control group).

Based on these results we devised a mathematical model used to forecast the risk of development of PPPID. According to the literature sources and our own observations we have picked 17 factors which were at the forefront of the aforementioned diseases and could stipulate a tendency to development of this complication and be potentially related to purulent-inflammatory diseases.

Such factors (Table 1) are as follows: surgery, extragenital and gynecological pathologies, complications during pregnancy and delivery, TORCH-infections, colpitis, fetal distress, invasive procedures during labour, changes in blood serum fluorescence characteristics etc. Quantitative measure of fluorescence intensity has been replaced to a qualitative one. This value will be "1" when patient's fluorescence intensity of blood serum ≤ 0.845, or "0" when fluorescence intensity of blood serum ≥ 0.845.

In terms of cohort formation, women were divided into 4 groups: under 18 years old (Group 0), 18 - 24 years old (Group 1), 25 - 34 years old (Group 2), and older than 35 years old (Group 3).

Table 1. Differences in anamnesis and clinical factors between the control group and main cohort (women with PPPID).

Factor	Control group (n = 40)	Main cohort (women with PPPID) (n = 170)	p
Surgery	12 (30%)	139 (81.8%)	<0.001
Extragenital pathology	7 (17.5%)	102 (60%)	<0.001
Gynecological diseases	12 (30%)	111 (65.3%)	<0.001
Complicated course of pregnancy	19 (47.5%)	143 (84.1%)	<0.001
Invasive procedures during labour	10 (25%)	89 (52.4%)	0.002
Risk of miscarriage	4 (10%)	40 (23.5%)	0.058
TORCH-infections	2 (5%)	74 (43.5%)	<0.001
Colpitis	7 (17.5%)	149 (87.6%)	<0.001
Labour duration > 12 hours	2 (5%)	12 (7.1%)	0.64
Vaginal tears during labour	9 (22.5%)	69 (40.6%)	0.03
Labour anomalies	1 (2.5%)	61 (35.9%)	<0.001
λ_{max} of blood serum	333.63±1.46	335.28±2.29	<0.001
Fluorescence intensity ≤ 0.845 в.о.	6 (15%)	152 (89.4%)	<0.001
Bed days	4.5±1.47	5.69±1.99	<0.001
Fetal distress	1 (2.5%)	36 (21.2%)	0.005
Risk of miscarriage + respiratory disease	2 (5%)	10 (5.9%)	0.82

We performed a stepwise logistic regression (with forward selection) in order to separate the factors, whose cumulative effect would have a significant effect on PPPID.

A probability of PPPID taking place (Q), depending on the selected factors was calculated using the following formula:

$$Q = \frac{1}{1+e^{-R}} * 100\% \quad (1)$$

where $e = 2.72\cdots$—is the base of a natural logarithm,

R—is the quantity calculated according to the Formula (2), mentioned below:

$$R = K + \beta_1 x_1 + \beta_2 x_2 + \cdots + \beta_n x_n \quad (2)$$

where K—a constant,

β_i—coefficients that correspond to a number of calculated factors,

x_i—corresponding numerical values of the factors.

Theoretically, Q can hold a value ranging from 0% (an impossible event) to 100% (a constantly occurring event). The meaning of β_i coefficients is calculated by the software and is represented by the natural log of the correlation of probabilities of corresponding variables. Increasing the value of the independent variable by a unit of measurement would increase the chances of developing complications in EXP (β) times.

The equation was evaluated according to Akaike information criterion [17], verification using χ^2 for the likelihood ratio test and by Nagelkerke's R2 (Pseudo R-squared) [18] [19]. Furthermore, ROC-analysis was used in order to determine a mathematical credibility of the model and calculate an optimal threshold of decision making (cut-off point).

As a result, a ROC-curve was implied in order to demonstrate correlation between specificity and sensitivity. The area under the curve (AUC) was calculated to characterise the model's quality, where the scale varied between 0.5 (method is unacceptable) to 1% - 100% which is an indication of the congruence in prognosis based on the model.

R-studio V1.1.442 was used to carry out statistical analysis, which was then exported for further analysis in MS Excel.

By using logistic regression, we were able to isolate 13 factors out of a total of 17, whose cumulative effect had the biggest impact on development of postpartum endometritis (**Table 2**).

The resulting model is correct with a probability of more than 99% ($p < 0.001$; $\chi^2 = 174.74$; df = 13). We determined that all 13 factors have high impact on development of PPPID, however the extent of this impact varies. EXP (β) is a factor that determines possibility of increased chances of complication arising when the independent variable increases. For instance, an extra bed day during postpartum period increases chances of the patient having postpartum purulent-inflammatory complications by 1.21 times. The biggest influence, however, is showed by a value ≤ 0.845 of fluorescence intensity of blood serum; this value increases chances of complication arising in 604 times. **Table 2** demonstrates results of calculated regression coefficients.

Table 2. The results of regression coefficients related to occurrence of PPPID in the main cohort (n = 170) using logistic regression.

Factor	β	Exp(β)	Variable	z	p
Constant	−78.1471			−0.72	0.472
Surgery	3.8457	46.79	V1	2.72	0.006
Extragenital pathology	2.5346	12.61	V2	1.94	0.053
Gynecological diseases	1.4007	4.06	V3	1.14	0.255
Risk of miscarriage	2.6075	13.57	V4	1.34	0.181
TORCH-infections	2.6978	14.85	V5	1.08	0.282
Colpitis	4.9746	144.69	V6	3.30	0.001
Labour > 12 hours	3.7835	43.97	V7	1.75	0.081
Labour anomalies	0.2017	1.22	V8	0.12	0.902
λ_{max} of blood serum	0.2012	1.22	V9	0.62	0.534
Fluorescence intensity ≤ 0.845	6.4036	603.99	V10	3.39	0.001
Age	0.4201	1.52	V11	0.41	0.685
Bed days	0.1896	1.21	V12	0.57	0.568
Fetal distress	2.7279	15.30	V13	1.08	0.281

By replacing β coefficient with data from **Table 2** in Equation (3) we can determine R and as a result, can forecast the probability of PPPID.

$$R = -78.1471 + 3.8457 * V1 + 2.5346 * V2 + 1.4007 * V3 + 2.6075 * V4 + 2.6978 * V5 + 4.9746 * V6 + 3.7835 * V7 + 0.2017 * V8 + 0.2012 * V9 + 6.4036 * V10 + 0.4201 * V11 + 0.1896 * V12 + 2.7279 * V13 \quad (3)$$

In Equation (3), qualitative factors are demonstrated as a bed day (independent variable V12) and as a max of the blood serum (independent variable V9). Age (independent variable V11) has four levels: 0 – under 18 y.o., 1 – 18 - 24 y.o., 2 – 25 - 34 y.o. and level 3 – over 34 y.o. The rest of the variables are dichotomic, which can be either "1" when the patients have the aforementioned diseases (undergone hospital treatment, TORCH-infections, colpitis or reduction in blood serum fluorescence intensity < 0.845 etc.) or "0" when they are absent.

Nagelkerke coefficient of determination demonstrates which part of dispersion of independent variable can be explained by dependent variable introduced to the model and it equals 0.9076. This therefore confirms that the set of variables explains around 90% of dispersion of dependent variable. The area under the curve (AUC) equals 0.99. ROC-curve, which demonstrates a mathematical model of occurrence of PPPID among the main cohort is depicted on **Figure 1**.

Determination of the optimal threshold of decision making (cut-off point) was done by calculating optimum cut-off value, which maintains equality be-

tween sensitivity and specificity. For this particular model, the threshold was 0.78, leading to a conclusion that a patient is highly likely to be affected if the risk of occurrence of PPPID is >0.78. Threshold of decision making (cut-off point) in relation to specificity, sensitivity and accuracy of the mathematical model for the patients with PPPID in the main cohort is depicted on Figure 2.

At this threshold value of the decision making (cut-off point), sensitivity is 96.47%, specificity is 97.50% (Table 3), likelihood ratio of the positive result (LR+) is 38.58, the likelihood of negative result (LR−) is 0.04, positive prognostic value (PPV) is 99.39% while the negative prognostic value (NPV) is 86.67%.

Table 4 and Table 5 demonstrate selected results of the spectral and fluorescent characteristics of blood serum of both main and control cohorts of patients and corresponding calculation of the probability of development of postpartum purulent-inflammatory diseases. Among the patients in the main cohort (Table 5), only 6 cases had a low likelihood of developing PPPID.

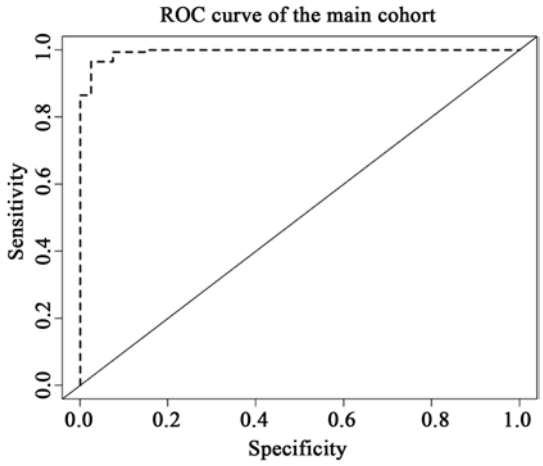

Figure 1. ROC-curve demonstrates a mathematical model of occurrence of PPPID among the main cohort.

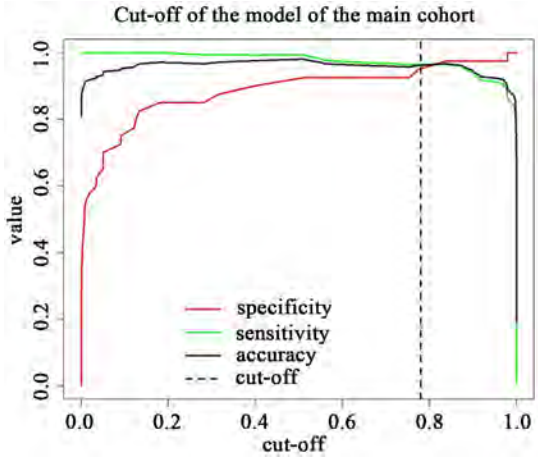

Figure 2. Threshold of decision making (cut-off point) in relation to specificity, sensitivity and accuracy of the mathematical model for the patients with PPPID in the main cohort.

Table 3. Diagnostic value of the mathematical model implied in prognosis of PPPID among new mothers in the main cohort.

	Control group (n = 40)	Main cohort (women with PPPID) (n = 170)	Total
Calculated value < 0.78	39 (97.5%)	6 (3.53%)	45
Calculated value ≥ 0.78	1 (2.5%)	164 (96.47%)	165
Total	40	170	210

Table 4. Probability of PPPID development in control group.

N°	Fluorescence intensity	λ_{max} of blood serum	Probability
1.	0.87	334.6	0.00124
2.	0.89	335.1	0.05022
3.	0.99	332.9	0.00259
4.	0.91	332.6	0.00751
5.	1	331.1	0.09102
6.	0.91	330.1	0.00043
7.	0.87	330.1	0.01822
8.	0.99	333.1	0.00339
9.	0.96	334.5	0.09032
10.	0.95	335.1	0.00056
11.	1	335.1	0.00949
12.	0.97	333.1	0.12568
13.	0.92	333.1	0.00076
14.	0.88	332.1	0.13294
15.	0.83	335.1	0.39702

Table 5. Probability of PPPID development in the main cohort.

N°	Fluorescence intensity	λ_{max} of blood serum	Probability
1.	0.55	334.1	0.99989
2.	0.58	336.1	0.99997
3.	0.61	343.1	0.99999
4.	0.67	336.8	0.91405
5.	0.77	333.3	0.95524
6.	0.63	338.1	0.99999
7.	0.51	337.1	0.99999
8.	0.53	339.1	0.99999
9.	0.57	343	0.99912
10.	1	336.1	0.65298
11.	0.88	333	0.526028
12.	0.89	336.1	0.51114
13.	0.77	332.8	0.55993
14.	0.73	335.1	0.180811
15.	0.71	336	0.54868

4. Conclusions

Within the scope of this investigation we implied fluorescent spectroscopy as the core method in the optimization of diagnostics of patients with postpartum purulent-inflammatory diseases. Based on 13 core factors of prognosis of postpartum purulent-inflammatory diseases (extragenital pathology, gynaecology-related diseases, risk of miscarriage, surgery, TORCH-infections, colpitis, pregnancy duration > 12 hours, anomalies during labour, λ_{max} of blood serum, fluorescence intensity ≤ 0.845, age, bed days, fetal distress) we have built a prognostic model of development of postpartum purulent-inflammatory diseases. Proposed model is correct with a probability of 99% ($p < 0.001$; $\chi^2 = 174.74$; $df = 13$). We determined that all 13 factors can provoke development of postpartum purulent-inflammatory diseases.

To sum up, it was understood that blood serum fluorescence intensity ≤ 0.845 is the largest contributing factor towards postpartum purulent-inflammatory diseases and has the biggest diagnostic value in evaluating the risk of development of these complications (including endometritis) among the main cohort of patients. We determined that according to our prognostic model only 6 patients (3.53%) of the main cohort (Table 3 and Table 5) had no risk of developing purulent-inflammatory diseases post labour.

Conflicts of Interest

Authors declare no conflict of interest regarding the publication of this paper.

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Research Progress of Heat Shock Protein 90 and Hepatocellular Carcinoma

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Abstract

Heat shock protein (HSP) is a kind of protein that mainly acts as a molecular chaperone to participate in the synthesis and folding of proteins, maintain the spatial conformation of proteins and protect cells from damage and other important biological functions. HSP90 plays an important role in maintaining molecular chaperone structure, regulating cell cycle and apoptosis, coordinating hormone signal transduction and promoting wound healing. And HSP90 also plays an important role in the occurrence and progression of tumors. In recent years, HSP90 inhibitors have made some achievements in molecular targeted therapy for malignant tumors, but further research is needed in clinical application. In this paper, the research status of the relationship between hepatocellular carcinoma targeted by heat shock protein 90 was reviewed.

Keywords

Liver Cancer, Heat Shock Protein 90, Molecular Chaperone, Inhibitor, Targeted Therapy

1. Introduction

Heat shock protein (HSP), also known as stress protein, is a kind of protein that is synthesized and expressed rapidly after the body is stimulated by external factors (high temperature, hypoxia, cytokine release, etc.) [1]. Heat shock protein (HSP) was first discovered in 1962 by Italian geneticist Ferruccio Ritossa [2] while studying the salivary gland chromosomes of Drosophila larvae. When the ambient temperature increases, the salivary glands of fruit flies bulge, which is called heat shock response (Heat Shock Response, HSR). Heat shock protein (HSP) is a large family of heat stress proteins that exist widely in bacteria, ani-

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mals and human beings. *In vivo*, it can mainly play the functions of "molecular chaperone", such as protein folding, transport, transmembrane, conformation stabilization, cell signal transduction, damage protection and so on [3]. According to the relative molecular weight, it can be divided into HSP27, HSP60, HSP40, HSP70, HSP90, HSP110 and so on. Heat shock protein 90 (heat shock protein 90, HSP90) is an important molecular chaperone to maintain the structural stability and function of many kinds of carcinogenic proteins in cells. It is highly expressed in hepatocellular carcinoma, and its substrate protein is a key signal molecule in biological behaviors such as growth and metastasis of hepatocellular carcinoma. HSP90 is closely related to the biological behavior of liver cancer, and it plays an important role in the occurrence, development, metastasis and prognosis of liver cancer [4]. This article reviews the mechanism and expression significance of HSP90 in the occurrence and development of hepatocellular carcinoma, as well as the research of HSP90 inhibitors in hepatocellular carcinoma.

2. Structure and Function of HSP90

The HSP90 family is a class of ATP-dependent molecular chaperones with a molecular weight of about 90 kDa. HSP90 exists in cells in the form of dimer, and the dimerization of HSP90 is necessary for its intracellular function. Its basic structure consists of three parts: an N-terminal domain (N-domain) that binds to ATP, an intermediate domain (M-domain) that connects the client protein, and a C-terminal domain (C-domain) that connects dimmers [5]. In addition, the HSP90, of eukaryotic cells has a charged region between the N-domain and the M-domain. The N-domain of HSP90 has a mold sequence structure of Bergerat folding, and the binding site region of ATP/ADP is a double-layer α/β sandwich structure. There are protein kinase B (PKB/Akt) binding sites in the M-domain of HSP90 and the main interaction sites between client proteins. They can also interact with auxiliary molecular chaperones and participate in the hydrolysis of ATP. The main function of M-domain is to bind to substrate peptides such as client proteins or other co-acting molecular chaperones, and its function may be different in the activation process of different types of customer proteins. The C-domain is a necessary region for HSP90 to form a dimer, which contains a highly conserved MEEVD amino acid sequence, which constitutes the core site for the binding of HSP90 to its chaperone tetratricopeptide repeat (TPR). The opening of the C-domain of eukaryotic HSP90 is closely related to the closure of the N-domain. The C terminal domain also contains a binding site between HSP90 and ATP, but the function of this site is not clear [6] [7].

HSP90 subtypes include cytoplasmic isoforms, HSP90a1/a2 and HSP90β, glucose regulated protein 94 (GRP 94) in endoplasmic reticulum and tumor necrosis factor receptor associated protein 1 (TRAP1) in mitochondria [8]. Human HSP90 is divided into two groups according to whether it contains rich glutamine fragments: HSP90α and HSP90β, which are composed of 730 and 724

amino acids respectively, and their homology is 84%. HSP90 exists mainly as α-α and β-β homodimers, each homodimer consists of two monomers, and each monomer includes the above three domains. In the non-stress state, the expression of HSP90 accounts for about 1%/2% of the total protein in the cell, which is thousands of times the average protein content; under the stress condition, the content of HSP90 can be increased to 4%/6% of the total protein in the cell [9]. The expression of HSP90α increases after heat induction, which is not necessary in mammals and is related to the maintenance of cell homeostasis under pressure, while HSP90β is sustainable and must exist in mammals, which is related to the life activities of mammals. These proteins are highly homologous in sequence, structure and regulation, and are collectively referred to as HSP90 in this review. As an important molecular chaperone, HSP90 can activate different substrate proteins and then participate in the regulation of a variety of life activities [10].

The main functions of heat shock protein 90 are: 1) molecular chaperone, HSP90 as molecular chaperone, which requires the binding and hydrolysis of ATP, different binding states with ATP/ADP can stabilize the activity of HSP90 substrate protein, promote the ubiquitination of substrate protein and degrade through proteasome pathway, 2) cell cycle regulation, HSP90 can regulate cell division from both positive and negative sides, promote cell division or inhibit cell division, 3) regulation of apoptosis, HSP90 can inhibit the formation of apoptotic bodies, regulate apoptosis, 4) coordinate hormone signal transduction, combine HSP90 with free steroid receptors (estrogen, progesterone, glucocorticoid, etc.) to maintain hormone stability and inactivity, 5) promote chronic refractory wound healing.

3. Expression of HSP90 in Tumor

HSP90 is highly expressed in a variety of tumors, which is closely related to the development and prognosis of tumors. It can interfere with tumor proliferation and metastasis by inhibiting HSP90, so it is a great potential antineoplastic drug at present.

HSP90 is highly expressed in breast cancer, which is related to the recurrence and lymph node metastasis of breast cancer. Studies have shown that the high expression of heat shock protein 90 is an independent prognostic factor for breast cancer and is significantly related to the survival time of breast cancer patients [11] [12] [13].

Anti-HSP90 studies have shown that it destroys EGFR-related signaling pathways, down-regulates the activity of HSP90, inhibits tumor cell proliferation, invasion and metastasis, and induces apoptosis of lung cancer cells [14]. HSP90 is highly expressed in lung cancer and promotes the proliferation and metastasis of lung cancer cells. In addition, the high expression of HSP90 in patients with Non small cell lung cancer (NSCLC) is related to shorter overall survival, suggesting that it can be used to predict survival, and the high expression

of HSP90 can be regarded as an independent prognostic factor of lung cancer [15] [16]. At present, there are a large number of reports about HSP90 inhibitors against lung cancer, but studies have shown that not all lung cancers respond to HSP90 inhibitors [17] [18]. The related mechanism of HSP90 regulating lung cancer at the molecular level still needs to be further explored.

It has been found that thyroid carcinoma (TC) is a tumor with high expression of HSP90. HSP90 is closely related to the occurrence, development and lymph node metastasis of TC [19]. The detection of the expression of HSP90 in TC is helpful to the diagnosis and prognosis of TC, and even can be used as a valuable reference index for differential diagnosis of benign and malignant thyroid lesions and evaluating the prognosis of TC. WHITE *et al.* [20] through the study of new HSP90 inhibitors, it was found that HSP90 inhibitors inhibit the invasion and metastasis of TC and its cancer stem cell (CSCs). When observing the efficacy of HSP90 inhibitor ganetespib in the treatment of thyroid cancer, Taiwan scholars found that the inhibitor effectively inhibited the proliferation of cancer cells [21].

It was found that berberine (BBR) combined with the second generation HSP90 inhibitor NVP-AUY922 produced a synergistic anti-proliferation effect on colorectal cancer cells sensitive to and insensitive to NVP-AUY922. Dual inhibition of HSP90 may enhance the efficacy of HSP90 inhibitors and overcome the drug resistance of cancer cells. HSP90-ERK-VEGF signaling pathway plays an important role in vascular endothelial cell migration and tubular formation induced by colorectal cancer.

HSP90 is highly [22] expressed in gastric cancer and participates in tumor cell proliferation, differentiation, metastasis and drug resistance. From gastritis to gastric cancer, the expression of HSP90 showed an increasing trend. In gastric cancer, HSP90 is mainly expressed in cytoplasm and a small amount in stromal cells. It is necessary to find the chaperone activity of HSP90 in the progression and invasion of gastric cancer. The high expression of HSP90 in gastric cancer provides an important basis for developing it as a diagnostic marker, therapeutic target and prognostic index [23].

Previous studies have shown that heat shock protein is highly expressed in pancreatic cancer and is closely related to the occurrence, development and metastasis of pancreatic cancer, but it is not a meaningful prognostic indicator of pancreatic cancer. Other studies have shown that HSP90 inhibitors combined with 2,2-difluorodeoxycytidine inhibit the normal growth of pancreatic cancer and completely inhibit tumor liver metastasis. Other studies have shown that HSP90 immunization can play an effective anti-tumor effect. And it can enhance the anti-tumor immunity of the body by stimulating the proliferation of T lymphocytes.

4. The Relationship between HSP90 and Hepatocellular Carcinoma

Hepatocellular carcinoma (HCC) is a common tumor of the digestive system,

which has a high degree of malignancy and poor prognosis. At present, studies have shown that HCC is a tumor with high expression of HSP90a. The increase of intracellular HSP90 expression is related to the occurrence and development of HCC, and may be an important sign of the development and deterioration of HCC. Using the molecular chaperone function of HSP, inhibiting the high expression of HSP, in HCC by DNA recombinant technique can improve the sensitivity of HCC to radiotherapy and chemotherapy, down-regulate the stability of oncoproteins related to the malignant proliferation of HCC, and then inhibit the proliferation of tumor cells, which is of great significance for the effective prevention and treatment of HCC.

There is a certain correlation between HSP90*α* and the biological characteristics of patients with liver cancer. Some studies [24] [25] have shown that the early recurrence and metastasis of patients with liver cancer after treatment are mostly related to the size of tumor focus, satellite focus, vascular tumor thrombus, microvascular invasion, degree of differentiation and pathological classification. It shows that there is a certain relationship between HSP90 *α* and the prognosis of patients. Studies have shown that [26], the prognosis of patients with HSP90*α* negative HCC is significantly better than that of patients with positive HCC ($P < 0.05$). Li Hui *et al.* [27] have shown that HSP90*α* is related to the metastatic potential of HBV-related HCC, and is expected to be one of the markers for HCC invasion and metastasis, prognosis evaluation and differentiation between HBV-related liver diseases and healthy people.

FU *et al.* [28] detected the plasma HSP90a protein level in 1652 cases of liver cancer and its control group by ELISA and found that plasma HSP90a could distinguish liver cancer from non-liver cancer patients (sensitivity 92.7%, specificity 91.3%), plasma HSP90 was more sensitive to distinguish hepatocellular carcinoma than alpha-fetoprotein (93.3%), and HSP90a could significantly distinguish between AFP negative and AFP localized liver cancer. The plasma HSP90 of patients with liver cancer in surgery and interventional therapy group was higher than that in liver cancer group before treatment. This study shows that HSP90a can be used as a marker for the diagnosis of liver cancer and can also be used to evaluate the therapeutic effect of patients with liver cancer. In 2016, the State Food and Drug Administration approved plasma HSP90*α* as a marker for liver cancer, and the kit has been approved for clinical use.

HSP90 targeted cancer therapy begins with the natural inhibitor geldanamycin (GM), which comes from *Streptomyces hygroscopicus*, showing its anti-proliferation effect by binding to the ATP binding site of HSP90 [29] [30]. However, because of its unstable structure and hepatotoxicity, it cannot be used further [31]. Another important natural inhibitor of HSP90 is free radical (RD), which comes from *Bornauden monosporium*. It inhibits tumor growth in vitro by attacking the core ATP binding pocket of HSP90, but has been proved to be ineffective in vivo because of its unstable structure [32]. To overcome these initial problems, genetically modified analogues have been developed. Two impor-

tant transgenic derivatives are 17-AAG (also known as tanespimycin or 17-allylamino-17-demethoxygeldanamycin) and 17-DMAG (also known as alvespimycin or 17-dimethylaminoethylamino-17-demethoxygeldanamycin). The first HSP90 inhibitor evaluated in clinical trials was 17-AAG in 1999 [33]. However, it cannot be used further because of its poor solubility and bioavailability. 17-DMAG shows strong anti-tumor activity and good water solubility, which makes it participate in various phase I clinical trials; however, dose-limiting side effects still exist [34] [35].

Finally, the most advanced and effective HSP90 inhibitors were developed as the second generation derivatives of RD. The first in this category is NVP-AUY922 (also known as luminespib or VER-2296), which shows strong preclinical and clinical efficacy [36] [37]. By far, the most promising second-generation synthetic HSP90 inhibitor based on resorcinol is ganetespib (STA-9090), which binds to the N-terminal ATP binding pocket of HSP90 and destroys the chaperone cycle. The strong anti-tumor activity of this HSP90 inhibitor has been transformed from preclinical success to several clinical studies [38].

AUY922, an inhibitor of heat shock protein 90, blocked the growth of hepatoma cells in vitro and induced apoptosis of hepatoma cells through caspase activation and β-catenin fragmentation. AUY922 also reduces tumor growth in vivo, indicating that HSP90 has become a promising therapeutic target for HCC. AUY922 inhibits the viability and proliferation of hepatocellular carcinoma cells, but has no effect on normal hepatocytes, and inhibits the activity of HSP90. AUY922 induces apoptosis of hepatocellular carcinoma cells by activating caspases, β-catenin fragmentation and inhibiting β-catenin-mediated transcriptional activity [39].

5. Outlook

HSP90 is closely related to the biological behavior of liver cancer, and it plays an important role in the occurrence, development, metastasis and prognosis of liver cancer. The increased expression of HSP90 in hepatocellular carcinoma provides an important basis for the development of diagnostic markers, therapeutic targets and prognostic indicators. Extensive research has been carried out on the development of specific HSP90 inhibitors. Many tumor-related signal pathways are blocked by HSP90 inhibitors, which are expected to become potential therapeutic targets to inhibit tumor progression. New HSP90 inhibitors have a stronger inhibitory effect on the growth of liver cancer cells, providing a new way for the treatment of liver cancer. Understanding the function of HSP90 and its molecular mechanism in liver cancer is very important for improving the diagnostic accuracy of liver cancer and developing more effective chemotherapeutic drugs. However, the research and clinical application of HSP90 inhibitors in anti-tumor research and clinical application need to be further developed in the future.

Conflicts of Interest

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

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Abbreviation Note List

HCC: Hepatocarcinoma
HSP: Heat shock protein
HSR: Heat shock response
N-domain: N-terminal domain
M-domain: Intermediate domain
C-domain: C-terminal domain
PKB/Akt: Protein kinaseB
TPR: Tetratricopeptide repeat
Grp94: Glucose regulated protein 94
TRAP1: Tumor necrosis factor receptor associated protein 1
TC: Thyroid Cancer
NSCLC: Non small cell lung cancer
CSC: Cancer stem cell
BBR: Berberine
GM: Geldanamycin
RD: Radicicol
17-AAG: Tanespimycin
17-DMAG: Alvespimycin
NVP-AUY922: Luminespib
STA-9090: Ganetespib

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Utilization of Extracorporeal Membrane Oxygenation for Pulmonary Toxicity Caused by Inhaled Synthetic Cannabinoid. A Harbinger of Future Complications Associated with Inhaled Cannabinoid Products

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Abstract

There has been a dramatic increase in medical complications related to synthetic cannabinoid (SC) use either by water pipe or vaping. The legalization of marijuana in an increasing number of states has also resulted in an increase in a number of complications related not just to marijuana, but in particular, to SC. As a result, there have been recent increased reports of acute pulmonary injury related to inhaled SC products. We describe that rarely endotracheal intubation with mechanical ventilation has been required to treat the acute respiratory distress syndrome (ARDS) and the diffuse alveolar hemorrhage (DAH) associated with the acute toxicity of SC inhalation. We describe the second reported case of successful utilization of mechanical ventilation and extracorporeal membrane oxygenation (ECMO) in order to treat acute pulmonary toxicity caused by SC inhalation by a water pipe. While the exact pathophysiology of these interesting and recent pulmonary complications is unknown, the recent increase in exposure to SC via water pipe systems and vaping suggests that there will be many more cases of patients that will require ECMO as a form of life-saving therapy.

Keywords

Synthetic Cannabinoid, Extracorporeal Membrane Oxygenation, Mechanical Ventilation, Water Pipe, Vaping, Pulmonary Toxicity

1. Introduction

Emergencies related to synthetic cannabinoids (SC) have increased recently in the United States [1] [2] [3]. The legalization of marijuana in states such as Nevada, Maine, Colorado, and California has increased accessibility of SC leading to the presentation of medical complications related to SC [4] [5]. The most common adverse presentations of SC use include nausea, vomiting, anxiety, psychosis, paranoia, and agitation [6] [7] [8] [9]. In addition, there are case series and case reports of stroke, hypertension, cardiac toxicity, and encephalopathy related to SC inhalation. Specifically, there has been a recent increase in reports of respiratory pathology such as acute respiratory distress syndrome (ARDS), diffuse alveolar hemorrhage (DAH), and chronic pulmonary findings associated with inhaled SC use [3] [7] [10] [11]. The acute and chronic findings of direct pulmonary toxicity do not include the depression of respiratory drive caused by SC [4] [5]. In addition to SC induced respiratory depression, there has been a recent increase in cases due to direct pulmonary toxicity not related to aspiration or infection [3] [7] [10]-[15]. In all the reported cases, alveolar hemorrhages developed within 48 hours after SC inhalation suggesting a temporal relation [7] [10]-[15].

Direct pulmonary injury by SC leading to the development of ARDS and DAH requiring endotracheal intubation has been reported infrequently [7] [10]-[15]. Failure to successfully treat respiratory insufficiency, ARDS, and DAH caused by SC with endotracheal intubation and mechanical ventilation is even rarer. The utilization of Extracorporeal membrane oxygenation (ECMO) to treat such a patient has been reported on only one occasion in abstract form [16]. In this first full case report we describe a 21-year-old woman who developed interstitial pneumonitis which required endotracheal intubation and immediate utilization of ECMO in order to ensure proper gas exchange.

2. Case Report

A 21-year-old African American female with no chronic medical problems or past surgical history presented to the emergency department with a 12-hour history of dyspnea and hemoptysis. The previous night she inhaled an undocumented amount of synthetic marijuana, known as K2, from a water pipe. Upon arrival in the emergency department, the patient's symptoms included dyspnea, hemoptysis, throat pain, central chest pain and fatigue. Social history was positive for infrequent inhalation of natural marijuana. The patient reports that this was her first exposure to SC in any form. She had no known allergies.

Initial set of vitals showed T 98.5˚F, BP 91/55, HR 99, SpO_2 40% - 60%, RR 28. Physical exam in the emergency department revealed a patient in severe distress with diminished breath sounds and diffuse rhonchi.

Chest X-ray revealed left lower lobe and right upper lobe infiltrates. CT Chest showed similar findings with more involvement of the left lung compared to the right (**Figure 1**). The arterial blood gases were PO_2 60 mm Hg, PCO_2 52.6 mm Hg, and pH 7.21. WBC was 23,000 × 10^9/L.

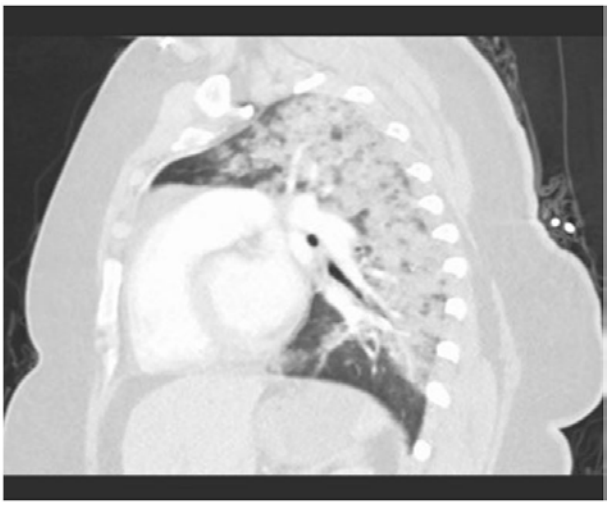

Figure 1. Sagittal CT chest pre-endotracheal intubation prior to initiation of ECMO demostrating diffuse intestinal edema.

The patient was rapidly decompensating, and a bag-valve mask was used before rapid sequence intubation. PEEP was set at 20 cm H_2O and the SpO_2 never exceed 60%. Despite these settings, she was unable to maintain adequate ventilation and pulse oximetry oxygen saturation remained in a range of 60% - 70%. At this time, the decision to initiate ECMO was made due to the inability to maintain adequate gas exchange and a 31 French Avalon veno-venous catheter placed (**Figure 2**). The patient was heparinized prior to initiation. Shortly after, she maintained hemodynamic stability with adequate gas exchange. She was decannulated on day seven, self-extubated on day eight, and discharged on day eleven. CT Chest prior to discharge demonstrated recovery of the acute lung injury (**Figure 3**). She made an uneventful recovery. Refer to **Table 1** for more information.

3. Discussion

The increased incidence of complications related to natural and synthetic cannabinoids has drawn great interest [17] [18]. In this recent time frame direct pulmonary toxicity caused by SC has also increased [10] [11] [13] [14] [15]. Our patient developed ARDS and DAH as defined by the Berlin criteria. Since our patient had bilateral ground glass opacities and bilateral air bronchograms that could not be explained by another condition, our patient conformed to the diagnosis of ARDS and DAH [19]. These opacities were not caused by cardiogenic pulmonary edema since the echocardiogram was normal. The patient had significant impairment of gas exchange. Medical history revealed the only risk factor for the development of ARDS and DAH in this patient was SC inhalation that night and late morning prior to admission.

Initial blood gases following endotracheal intubation revealed profound hypoventilation despite high level of pulmonary compliance. During endotracheal intubation, pink, frothy sputum emanated from the trachea. Although

Table 1. Sociodemographic information and clinical presentation of the patient.

Sociodemographics	**Age**: 21 y/o **Gender**: Female **Onset**: SC use 12 hours before admission **Previous SC Use**: Patient denies
Clinical Presentation	**Temperature**: 98.5 Fahrenheit **Blood Pressure**: 91/55 mm Hg **Heart Rate**: 99 beats per minute **SpO_2**: 40% - 60% after rapid sequence intubation **Respiratory Rate**: 28 respirations per minute **Blood Gas:** pO_2 60 mmHg, pCO_2 52.6 mmHg **pH**: 7.21 **Lactate:** 3.8 mmol/L **Procal:** 0.43 ug/L **Sed rate:** 23 mm/hr **Influenza:** Negative **BUN:** 13 mg/dL **Creatinine:** 0.74 mg/dL **Glucose:** 136 mg/dL **CO_2:** 24 mm Hg **Chloride:** 104 mEq/L **Potassium:** 3.8 mEq/L **Sodium:** 139 mEq/L **Leukocyte Count**: 23,000 × 10^9/L **Base Deficit:** 7 **Echocardiogram**: Negative **Clinical Observations**: Acute respiratory distress with frothy pink sputum arising from airway during intubation
ECMO and Ventilation Settings	**Initial Ventilation Settings (prior to ECMO):** FiO_2: 100% PEEP: 20 Respiratory Rate: 14 breaths per minute Tidal Volume: 400 ml SpO_2: Never exceeded 60% **Ventilation Settings (on ECMO):** FiO_2: 40% PEEP: 5 cm H_2O Respiratory Rate: 8 breaths per minute Tidal Volume: 400 ml **ECMO Settings:** 31 French Avalon veno-venous catheter Rate: 4.56 L/min FiO_2: 100% Sweep: 3.5 L/min Mixed Venous: 77.1% Activated Clotting Time (ACT): Above 200 Duration: 7 days
Time to Clearance of Images	15 days from admission to final CT Chest (**Figure 2** & **Figure 3**)
Complications	None, patient made an uneventful recovery

PEEP and pressure support settings of mechanical ventilation were high, this patient was unable to ventilate and maintain oxygenation adequately. Therefore, it was determined that this patient should be placed on VV-ECMO which was done within one hour following endotracheal intubation. Endotracheal intubation was not associated with aspiration.

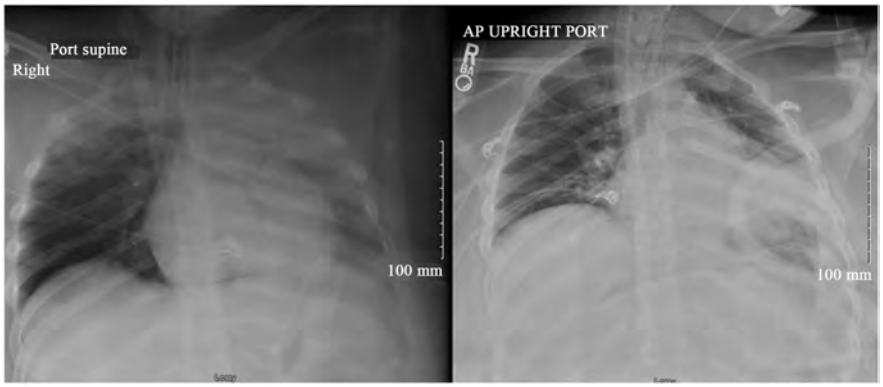

Figure 2. Chest X-rays immediately post placement of 31 French Avalon veno-venous catheter for ECMO.

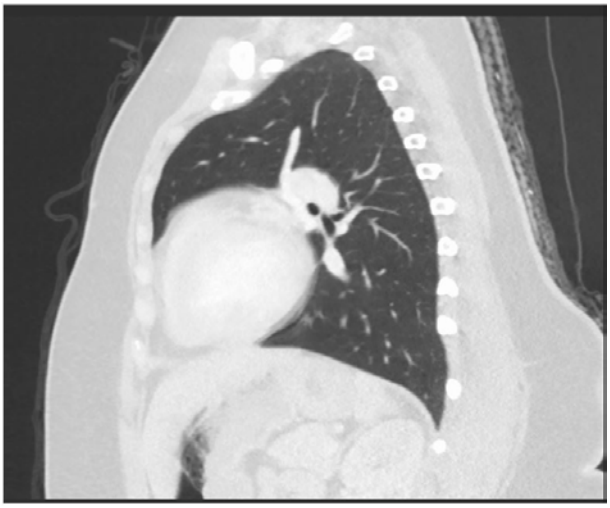

Figure 3. Sagittal chest CT post-ECMO, post-extubation showing recovery prior to discharge.

There were gradual improvements in the patient's condition following placement on VV-ECMO. The patient made an uneventful recovery, improving both clinically and radiographically.

Few case reports of diffuse lung injury caused by SC requiring mechanical ventilation have been found [10] [11] [13] [14] [15]. Many reports have focused on the depressive effects of SC on respiratory drive [12]. In these patients chest x-rays were unremarkable because the effect of SC was on the central nervous system's control of respiratory drive rather than the lung parenchyma itself. Since 2019, few reports of SC associated diffuse lung injury requiring mechanical ventilation have been described [10] [11] [13] [16]. Of these, only one utilized ECMO as a form of life-saving bridge therapy [16]. Review of the other cases demonstrates similar bilateral alveolar infiltrates, one in which was noted to be secondary to diffuse alveolar hemorrhage [10] [11] [13] [14] [15]. There has only been one reported case of SC inhaled by water pipe method [10]. Our patient used a water pipe similar to the bucket method as described in Yamanoglu, *et al*., 2018 [10]. It has been proposed that a higher concentration of the heterogenous

toxic metabolites from SC may cause a more direct injury to the lungs with this method of inhalation. In fact, synthetic marijuana potency relative to THC can be up to 660 times greater [20]. In this particular case, a similar bloody and pink, frothy aspirate suggests acute lung injury not caused by other entities [10].

This patient remained on ECMO for four days in our care with gradual clinical improvement. She was then transferred to an academic center where, after three more days, was decannulated and then self-extubated the next day. The patient was discharged one week later and made an uneventful recovery.

Acute pulmonary injury caused by SC inhalation is a rare event but is expected to become more common [7] [21]. This case demonstrates the severe life-threatening and rapid deterioration that may ensue after smoking concentrated SC through a water pipe.

Immediate recognition of impending respiratory insufficiency with the subsequent need for endotracheal intubation and provision of ECMO for continued failure of adequate ventilation and gas exchange must remain an immediate priority. With the recent increase in complications of SC reported recently, one would expect there will be many more cases to come.

Radiologic patterns characteristic of SC-induced pulmonary toxicity can be defined as diffuse, acute patches of alveolar infiltrates with patchy air bronchograms. This classic radiologic presentation is a function of bronchial endothelial injury which often presents as a diffuse centrilobular nodule with tree-in-bud pattern [7]. This response results in a pattern of injury consistent with organizing pneumonia.

Histopathologic findings of organizing pneumonia are also characteristic in patients with chronic SC associated lung injury [14]. CT radiograph may show a diffuse miliary-micronodular pattern and Chest CT may demonstrate diffuse centrilobular nodules and tree-in-bud pattern [7]. For patients who present with unexplained pulmonary infiltrates as described above, SC use should be included on the list of differential diagnoses. An appropriate history must be taken, and drug screen testing may be indicated.

Little is known regarding the etiology DAH associated with SC. In some cases, toxic metabolites have been linked to direct alveolar or bronchopulmonary injury leading to DAH [3]. These reported metabolites have been found to vary in chemical makeup and concentrations, resulting in a range of heterogeneous effects and potency. Testing for these cannabinoids is imperfect. ELISA is limited by the number of detectable metabolites and is not commercially available. Recently, a case of DAH associated with the SC metabolite UR-144, UR-144 N (4/5-hydroxypentyl) has been published [16]. Because of the direct toxic injury, steroids have been administered and may be an effective measure. Further investigation is needed.

Cannabidiol (CBD) inhalation products have become increasingly available. Common routes of inhalation include water pipes and electronic delivery (vaping) [22]. Recent reports from lay press have raised concerns that vaping CBD could be more detrimental when compared to water pipe inhalation due to un-

known additives such as SC in the vaping solution [23].

ECMO has proven to be crucial in the management of nicotine vaping related pulmonary injuries [24]. With recent CBD vaping trends in combination with an unregulated market, early anticipation and intervention with ECMO may be crucial and possibly lifesaving going forward.

4. Conclusion

Acute pulmonary toxicity causing severe injury and requiring mechanical ventilation is an uncommon phenomenon. We present a case of acute respiratory distress syndrome in a 21-year-old female whose only risk factor for developing sudden hemorrhagic pulmonary edema was the recent inhalation of SC through a water pipe. The etiology of this injury is not certain and would be challenging to determine due to the numerous pneumotoxic metabolites potentially found in SC. The immediate deterioration of this patient and her dramatic improvement following ECMO suggest that early consideration of ECMO for patients with respiratory insufficiency caused by acute pulmonary injury related to SC should be considered. Indications for ECMO are evolving. Regardless of etiology, the inability to ventilate and exchange gas, particularly in a young patient without multiorgan failure, necessitates early utilization of ECMO [25] [26].

Author Contributions

Authors have either participated in the care of the patient and/or the preparation of the manuscript and have approved of the manuscript.

Conflicts of Interest

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

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The Application of Laparoscopic B-Ultrasound Microwave Ablation Technology in Liver Metastasis of Colorectal Cancer

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Abstract

Liver is the most common metastasis target organ in the late stage of colorectal cancer. More than 50% of colorectal cancer patients will have simultaneous or heterochronous liver metastasis. The survival time of patients with colorectal cancer and liver metastasis (CRLM) is short; not all patients can get radical resection of liver metastasis. For this part of patients, microwave ablation technology has been proved to be one of the effective methods for the treatment of liver metastasis. Laparoscopic B-ultrasound ablation also highlights a lot of minimally invasive advantages; this paper reviews the relevant literature of PubMed database, Wanfang database and CNKI database, in order to provide the treatment basis for clinical application of microwave ablation technology under laparoscopic B-ultrasound in the treatment of CRLM. The results showed that the safety and effectiveness of microwave ablation for liver metastases under the location of B-ultrasonic laparoscopy were confirmed, and patients with liver metastases of colorectal cancer who could not be resected could choose this treatment.

Keywords

Laparoscopic Technique, Microwave Ablation, Colorectal Cancer Liver Metastases, Review

1. Introduction

Colorectal cancer is one of the most common malignant tumors in China, and liver is the most important target organ for its metastasis. For patients with colorectal cancer and liver metastasis (CRLM), radical resection of liver metastasis

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is still the gold standard, but 80% - 90% of patients with liver metastasis have no chance of radical operation. The survival time of patients with liver metastasis was significantly different between the two groups. It is very important to choose the local treatment for CRLM. Thermal ablation is divided into radiofrequency ablation, microwave ablation, laser ablation, high intensity focused ultrasound ablation and cryoablation [1]. Microwave ablation has been proved to be a safe and effective alternative for patients with liver cancer who are not suitable for surgical resection [2]. Some scholars [3] have proved that compared with other non-radical hepatectomy treatments, microwave ablation is a more safe and effective treatment for liver metastasis in CRLM patients. With the development of microwave ablation technology and the development of minimally invasive surgery and the concept of accelerated rehabilitation surgery, microwave ablation technology has been applied in the treatment of CRLM in recent years, and the effect is accurate.

2. Theoretical Basis of Microwave Ablation under Laparoscope

1) Principle of microwave ablation under laparoscope

Microwave ablation is one of the local thermal ablation techniques, which uses high frequency electromagnetic wave to produce heat on tissue and make tumor cells produce thermal coagulation. Polar molecules are the material basis of heat generated in living tissue by microwave ablation. The common frequency of microwave ablation for tumor treatment is 915 and 2450 MHz. In this microwave field, polar molecules such as water molecules and protein molecules rub with each other, part of kinetic energy is converted into heat energy, so that the tissue is heated. Compared with normal cells, tumor cells have poor heat tolerance. Under the action of high temperature, tumor cells coagulate and necrosis, so as to achieve the purpose of treatment [4]. In order to place the tumor tissue in the microwave field, the surgeon uses the microwave antenna to bury the tumor tissue. Under the B-ultrasonic image, the liver space occupying lesions can be manifested as uneven low, equal, high echo or mixed echo, and there is often complete or incomplete low echo around the tumor, with obvious characteristics. The microwave antenna can be directly buried in the liver lesions under the B-ultrasound location. This treatment scheme has been proved to be safe and effective [5]. In the study of microwave ablation of liver cancer, many scholars [6] have proved that ≤5 cm of liver cancer can achieve therapeutic effect in recent years. The clinical application of laparoscopic technology is now very mature, and the basic hospitals are gradually developing. Most of the elderly and patients with some basic diseases can bear it. With the development of endoscopy technology and microwave ablation technology, the combination of them can expand the treatment of liver metastasis of colorectal cancer.

2) Advantages of microwave ablation under laparoscope

Since the introduction of radiofrequency ablation in 1990, local ablation technology has been gradually applied in the treatment of liver tumors. Com-

pared with other thermal ablation treatments, microwave ablation has its own advantages. Compared with radio-frequency ablation, it has the characteristics of fast heating, high temperature in tumor, short time, little influenced by carbonized blood flow and no influence by impedance, so it has made great progress in clinical. Some scholars have pointed out that compared with radio-frequency ablation, microwave ablation has lower cost and more advantages for large tumors [7]. In the microwave ablation of liver lesions, it can be divided into percutaneous microwave ablation and laparoscopic microwave ablation, which have received good clinical effect [8] [9]. Compared with the two, laparoscopic microwave ablation can give full play to the advantages of direct vision. It can not only avoid unnecessary side injuries, but also find no lesions that are difficult to be found through auxiliary examination before operation, so that the effectiveness of microwave ablation can be guaranteed [10]. According to related studies, different ablation methods, such as laparotomy, laparoscopy and percutaneous liver tumor ablation, can affect the local recurrence of the tumor. Kuvshinoff *et al.* [11] have studied that laparoscopic tumor ablation has a lower local tumor recurrence rate than percutaneous puncture. Laparoscopic technology in microwave ablation is not only the role of direct vision or exploration, but also combined with other treatment programs to play its advantages.

3. The Application of Microwave Ablation under Laparoscope in CRLM

1) Microwave ablation of B-ultrasonic localization under simple laparoscope

When colorectal cancer patients find liver metastases, they seldom get the opportunity of operation. Effective local treatment is particularly important for CRLM. In clinical application, endoscopic technology has been very mature, most patients can tolerate, laparoscopic direct vision of thermal ablation can try to avoid the side injury of ablation treatment, but also can achieve good ablation effect. With the continuous improvement of minimally invasive technology and the continuous progress of ablation technology, under the common requirements of ensuring effective ablation rate and rapid rehabilitation, the results of laparoscopic B-ultrasound-guided thermal ablation for CRLM are satisfactory. Many scholars [12] [13] have pointed out that it can be used as a local treatment method for non resectable liver metastases. The application of laparoscopic ultrasound system enables surgeons to observe not only the surface of tissues and organs, but also the internal conditions of operation field in real time and from multiple angles. Sun Wenyu *et al.* [14] showed that for the liver metastases with multiple lesions, microwave ablation was performed on the intrahepatic lesions under the ultrasonic positioning first, and for the lesions with special location invading the hepatic capsule, microwave ablation was performed on the base of the lesions to cut off the blood supply of the tumor completely; the ablation rate was 84.6% in the reexamination one month after the operation; it was proved that the microwave ablation guided by ultrasound under the laparoscope was not suitable for the unresectable lesions Liver metastasis of colorectal cancer is a safe

and effective treatment. Luo Hongchang *et al.* [15] in 2015, 71 cases of HCC with cirrhosis were treated by microwave ablation under the guidance of laparoscopic ultrasound, with good results. Only one patient had serious postoperative complications (1.4%) and no death. One month later, the reexamination showed that the liver lesions were completely ablated and no residual was found. After a follow-up of (11.5 ± 10.1) months, local recurrence was found in 4 lesions, distant metastasis in 6 lesions, and local recurrence and distant metastasis in 3 lesions simultaneously. Laparoscopic ultrasound-guided microwave ablation of liver cancer has the advantages of safety, effectiveness, short recovery period and low incidence of complications. Liu Wenbin *et al.* [16] conducted a comparative analysis of laparoscopic ultrasound-guided and open-ended ultrasound-guided microwave ablation. It was found that patients with laparoscopic treatment had less bleeding, shorter time to recover gastrointestinal function and shorter time to stay in hospital after surgery, while there was no significant difference in operation time, focus ablation time, hospitalization cost and complete ablation rate of focus. Some scholars have proved that the complete ablation rate of tumor is better than that of the percutaneous treatment group, not only minimally invasive but also accurate, and the postoperative recovery of patients is faster [17]. In the study of therapeutic devices, some scholars [18] [19] have made use of pulse microwave and mtDNA targeting single-walled carbon nanotubes as microwave absorbers for the ablation of liver tumors. They have also reached safe and effective conclusions. Ziemlewicz TJ *et al.* [20] used high-power, air-cooled system and multi-needle combined positioning therapy in clinical application to prove its technical achievements and device safety. Zafar T. *et al.* [21] proposed that slot antenna combined with microwave analysis is a reasonable choice for microwave ablation, with low cost and simple design; it can analyze the internal temperature distribution curve of malignant tissue, so that the operator can understand the ablation effect. In terms of improving the accuracy of liver metastases under laparoscope, surgeons and ultrasound doctors have also conducted various studies. Yan Shi Yan *et al.* [22] compared the treatment of real-time contrast-enhanced ultrasound with that of standard ultrasound, and the real-time contrast-enhanced ultrasound under laparoscope is more accurate in positioning liver lesions. There are also some scholars who use MRI [23], PET-MRI [24], robot guided [25] to locate the focus for treatment, but the clinical application is not extensive. Wu Wenbo *et al.* [26] use the biological simulator to simulate the human body so that the surgeon can carry out surgery practice, which can not only enable the surgeon to understand the operation scheme for surgery simulation, but also shorten the learning curve.

2) Laparoscopic ablation combined with other therapies

a) Combined operation

As the safety and effectiveness of thermal ablation have been gradually confirmed, minimally invasive surgery has gradually matured in clinical practice; laparoscopic ultrasound-guided thermal ablation combined with minimally invasive surgery makes some unresectable liver metastases to be treated, and in

clinical practice, it is also increasingly favored by the surgeons, retaining more normal liver, so patients benefit. Eng Oliver s *et al.* [27] conducted a retrospective cohort study. They analyzed the curative effect of laparoscopic radical resection of colorectal cancer combined with radiofrequency ablation in the treatment of liver metastases and that of open radical resection of liver metastases of colorectal cancer. They found that there were statistical differences between the two groups in the comparison of operation time, bleeding volume of the operator, overall postoperative complications and postoperative hospital stay, which also confirmed the laparoscopic operation. The advantages of lower microwave ablation combined with minimally invasive surgery are less complications, faster recovery and better prognosis. Diao Jingfang *et al.* [28] pointed out that microwave ablation combined with simultaneous operation is safe and effective for liver metastases with diameter ≤ 3 cm and number ≤ 3. It is not only the application of thermal ablation in radical resection of colorectal cancer, but also in hepatectomy. Some scholars have proved the effectiveness of microwave ablation combined with hepatectomy in the treatment of HCC and liver metastasis. For the liver lesions with special location or single small lesions, not only enough liver should be reserved, but also the standard of R0 resection should be met. The combination of hepatectomy and microwave ablation has played its advantages [2] [29].

b) Combined with other treatments

Si Zeng Mei *et al.* [30] used microwave ablation under laparoscope combined with TACE to treat liver cell carcinoma ≥ 5 cm, which proved that it was also safe and effective. Zhao P *et al.* [31] used TACE combined with microwave ablation to treat primary liver cancer has reliable safety and effectiveness. It is also safe, reliable and easy to operate to use microwave frequency ablation combined with TACE for liver metastases with special location or difficult to remove. Chen Kaiyun *et al.* [32] used laparoscopic ablation combined with 125I particles to treat liver metastases, and successfully treated the newly found lesions during the operation. The 1-year survival rate and 2-year survival rate of 62 patients were 74.2% and 59.7%, respectively. Beerman Marie *et al.* [33] concluded in 1000 cases that laparoscopic ablation combined with CT imaging computer-aided targeting technology is very successful, and there is no obvious learning curve, and there is less side injury. The research of Yasunori Minami *et al.* [34] shows that there is a potential effect of heat ablation on immunosuppressive sites of liver metastases, and combined immunotherapy is also expected to become a new direction. The combination of laparoscopic thermal ablation and other treatment schemes has better effect, safety and reliability, and will produce more effective treatment schemes in the future.

4. Summary and Outlook

The safety and effectiveness of laparoscopic thermal ablation for primary liver cancer and colorectal cancer with liver metastasis have been gradually con-

firmed, and it is also one of the effective treatments for colorectal cancer with liver metastasis to achieve R0 resection. Compared with laparotomy and percutaneous ablation, laparoscopic thermal ablation has lower local recurrence rate, less bleeding and shorter hospital stay. The learning curve can span in about 30 cases and is gradually applied in clinical practice. However, most of the conclusions are from retrospective analysis or single center research, which requires more prospective experiments or multi-center clinical experiments, so as to reduce disputes and let patients benefit better. Laparoscopic microwave ablation combined with other treatment schemes also provides a new idea for comprehensive treatment. Multidisciplinary comprehensive diagnosis and treatment is the standard treatment scheme for colorectal cancer patients, and reasonable selection of patients for appropriate treatment is the guarantee of patients' benefits.

Conflicts of Interest

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

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Comparative Study on Malaria Preventive Practices among Under-Five Children in Three States in South-South Nigeria

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Abstract

Background: Malaria has remained one of the leading causes of morbidity and mortality in children despite effective preventive and treatment modalities. This study is aimed at looking at the malaria preventive practices among under-five children in three Niger Delta states in Nigeria and comparing the differences among them if any. **Methods:** This was a cross sectional study carried out over six months from 1st January to 30th June 2019 in public health facilities among under-five children in three South-South states (Akwa Ibom, Delta and Rivers) of Nigeria. Using a stratified sampling method, children were recruited from 36 health facilities in the three states. A pretested interviewer administered questionnaire was used to harvest relevant information on socio-demographic characteristics of the subjects and informants and malaria preventive practices. Obtained data was analysed using SPSS version 22 and results are presented in prose and frequency tables. Chi-square and Fischer's exact were used for comparison of categorical variables, while a p-value of <0.05 was considered statistically significant. **Results:** A total of 3144 children participated in the study: 1661 (52.8%) were males while 1483 (47.2%) were females. Children less than 2 years represented 77.6% of the study participants while the mean age was 1.72 ± 1.06 years. Mothers constituted over 80% of the informants in all the states. More of the informants had

secondary education in Akwa Ibom and Delta states, while in Rivers state, more of them had tertiary education. Malaria prevention method practiced in the three states included; use of insecticide treated bed net (ITNs), insecticide spray, anti-malarial drugs, clearing of bushes and disposal of mosquito breeding cans and use of mosquito repellents. Indoor residual spraying (IRS) was not practiced in any of the states. Use of ITN was practiced more in Rivers state (53.2%) than in Delta (20.3%) and Akwa Ibom (8.2%) states, while use of insecticide spray was commoner in Akwa Ibom state (77.2%) than in Delta (56.3%) and Rivers (42.4%). This difference in the prevention techniques practiced among the states was statistically significant (Fischers exact −724.2, p-value = 0.0001). **Conclusion:** In conclusion, the practice of ITNs use is low in South-South Nigeria with IRS not being practiced at all. Introduction of IRS as a method of malaria vector control and public health education on ITNs ownership and use is advocated.

Keywords

Malaria, Preventive-Practices, Under-Five-Children, South-South Nigeria

1. Introduction

Malaria is a protozoan infection in humans caused by the parasite Plasmodium. It is a life-threatening vector borne infection and children under 5 years and pregnant women are the most vulnerable group in endemic areas. Malaria is essentially a disease of the tropics and subtropics particularly the sub-Saharan African region although it can be found in temperate areas due to migration from the tropics. Over 90% of the world's malaria occurs in sub-Saharan Africa and about 500 million cases are recorded annually; a child under the age of 5 years is said to die from malaria in every 2 minutes [1] [2]. Malaria has continued to be one of the leading causes of morbidity and mortality in children despite effective preventive and treatment modalities and more than 20% of world's population are affected by malaria [3]. The morbidity and mortality from malaria are still unacceptably high in the developing countries, especially among the vulnerable group, despite all control efforts.

Malaria remains a public health problem facing developing countries and Nigeria being the most populous African country bears a very high percentage of this burden. Nigeria accounts for 25% of global malaria burden [4] and being the eighth most populous country in the world, the burden of malaria in Nigeria is conversely a world burden especially with the level of migration of Nigerians particularly to the western world. The UK Health Protection Agency [5] states that about 1614 malaria cases were reported in the UK yearly between 2005 and 2010, and 22% of these cases in 2010 were from Nigeria. Estimate shows that over 50% of Nigerians suffer at least about of malaria every year [3]. And malaria accounts for 20% and 25% of under-five mortality and childhood mortality re-

spectively and this constitutes a huge economic burden [1] [6] [7].

Malaria is holo-endemic in Nigeria with transmission all the year round. Nigeria has two main seasons in the year, the dry season which is from October to about March and the rainy season which is from April to September with peak of the rains between May and July when malaria transmission is very intense. Rainfall pattern in Nigeria varies with the South having more rains than the North. Annual rainfall decreases northward; rainfall ranges from about 2000 millimeters in the coastal zone (averaging more than 3550 millimeters in the Niger Delta) to 500 - 750 millimeters in the north. The far south is defined by its tropical rainforest climate, where annual rainfall is 60 to 80 inches (1524 to 2032 mm) a year. The Niger Delta is located on the Atlantic coast of Southern Nigeria encompassing an area of 20,000 km^2 and it is the world's third largest wetland [8]. The mangrove swamp forest vegetation encourages all-year-round transmission of malaria in this area.

The knowledge of the preventive measures of malaria is an important preceding factor for the acceptance and use of malaria preventive practices [9]. Surveys of residents of the Atlantic coast revealed a lack of knowledge and many misconceptions about the transmission and treatment of malaria, which could adversely affect malaria preventive practices and treatment [3] [10]. Prevention of malaria is currently based on two complementary methods: chemoprophylaxis especially in pregnant women and protection against mosquito bites. While several malaria vaccines are under development, none is available yet. Measures aimed at protection against mosquito bites include use of personal protection measures against mosquito bites and vector control measures. These include use of insecticide treated bed nets (ITNs) especially long-lasting insecticidal nets (LLINs), indoor residual spraying (IRS), use of protective nets in windows, doors, ventilators, and eaves to prevent access of mosquitoes, larviciding, and clearing vegetation and containers around houses where mosquitoes harbour and breed [11] [12] [13].

Assessing malaria preventive practices in the high rainfall region of the Niger Delta will ascertain the local malaria situation and help to develop appropriate control measures to advice policy makers and the residents in the subregion with appropriate knowledge of the health problem [1]. This study therefore is aimed at looking at the malaria preventive practices among under-five children in the three Niger Delta states and to compare the differences among them if any.

2. Methodology

This was a cross sectional study carried out over a six month period from 1st January to 30th June 2019 in public health facilities in Akwa Ibom, Delta and Rivers states. It is the second arm of the first phase of the Niger Delta Development Company (NDDC) sponsored malaria research on its burden and preventive practices among pregnant women and under-five children being carried out in the nine South-South states of Nigeria. Sample size was calculated using the formula for comparison of groups [14] given as a minimum of 100 under-five

subjects to be recruited per study site in the Niger-Delta states. The sample size calculation for the study took into account an attrition rate of 10%. Using a stratified sampling method, each of the three states was stratified into three senatorial districts via equal allocation giving a total of nine senatorial districts. In each of the senatorial district, a list of health facilities involved in the care of children constituted the sampling frame. Simple random sampling via computer generated table of random numbers was used to select four health facilities from the sampling frame from each senatorial district giving a total of 12 health facilities from each state and a total of 36 health facilities from the three states. In each study centre, the study was carried out in the Department of Paediatrics within one to two weeks. The first 100 consecutive children who are less than 5 years and whose parents or guardian gave consent for the study were selected. A pretested interviewer administered questionnaire was used to harvest relevant information on socio demographic characteristics of the subjects and informants (age, sex, weight, height, education and occupation), and malaria preventive practices [insecticide treated bed net (ITNs) use, Indoor residual spraying (IRS), antimalarial medicine use, use of mosquito repellents, clearing of bushes and disposal of mosquito breeding containers, presence of window nets in homes]. Obtained data were analysed using SPSS version 22 and results are presented in prose and frequency tables. Chi-square and Fischer's exact was used for comparison of categorical variables, while a p-value of <0.05 was considered statistically significant.

Ethical Consideration

Ethical clearance for the study was obtained from the Research and Ethics committee of the University of Port Harcourt Teaching Hospital (UPTH) before proceeding with the study. Notification and permission to carry out the study was obtained from the Ethics Committee or hospital administration of selected hospitals.

Informed Consent

All parents and guardians of participating children were informed on all aspects of the study and provided a written informed consent. They were also informed that they were free to withdraw their children from the study at any time.

3. Result

Sex and Age distribution of the children from the three states:

A total of 3600 children were recruited for the study, 456 had incomplete and inconsistent data and were excluded in the final analysis. A total of 3144 children participated in the study giving a response rate of 87.3%. Out of these, 1661 (52.8%) were males while 1483 (47.2%) were females. Children less than 2 years represented 77.6% of the study participants while the mean age was 1.72 ± 1.06 years. Table 1 shows that there was a near equal proportion of the male and female participants across the three states with no statistically significant difference (X^2 = 2.66, p = 0.2642). However, there was a statistically significant

Table 1. Sex and age distribution of the children from the three states.

State	Akwa Ibom	Delta	Rivers	Total	Chi-square/P value
Variable	**No (%)**	**No (%)**	**No (%)**	**No (%)**	
Sex of child					
Male	506 (50.7)	542 (53.8)	613 (53.9)	1661 (52.8)	2.66 (0.2642)**
Female	492 (49.3)	466 (46.2)	525 (46.1)	1483 (47.2)	
Age of Child					
<2 years	805 (80.7)	756 (75.0)	879 (77.2)	2440 (77.6)	**9.38 (0.0092)***
>2 <5 years	193 (19.3)	252 (25.0)	259 (22.8)	704 (22.4)	
Mean age	1.64 ± 0.99 years	1.78 ± 1.10 years	1.74 ± 1.08 years	1.72 ± 1.06 years	**0.0009***

*Distribution is statistically significant ($p < 0.05$); **Distribution is not statistically significant ($p > 0.05$).

difference in the different age groups and the mean age of the participants across the different states ($X^2 = 9.38$, $p = 0.0092$).

Survey Informant (Respondent)

The modal informant across the three states was the mothers and they constituted over 80% of the informants in all the states. However, comparing the different informants from the different states showed a statistically significant difference, for example fathers constituted 7.6% of the informants in Rivers state and only 1.5% in Delta state (Table 2).

Parents' Educational Level

Table 3 shows that in Akwa Ibom state, more of the participants had obtained secondary education, among the fathers, there were 492 (49.7%) and 486 (48.9%) among mothers; also, in Delta state, more of the participants had obtained secondary education, 559 (56.6%) and 572 (57.1%) among the fathers and mothers respectively. However, in Rivers state, more of the participants had obtained tertiary education; among the fathers, there were 697 (61.8%) and 605 (53.4%) among mothers. These differences in the educational level of the parents were statistically significant when compared among the states. ($X^2 = 210.6$, p-value = 0.0001).

Social Class (Oyedeji's Social class classification) [15]

Distribution of respondents by social class reveals that the middle social class was most represented in Akwa Ibom and Delta states, constituting 617 (61.8%) and 617 (61.3%) respectively. In Rivers state, the upper and middle social class were most represented, constituting 560 (49.4%) and 548 (48.3%) respectively. This difference in social class among the respondents across the three states was statistically significant ($X^2 = 175.6$, $p = 0.0001$) (Table 4).

Family's monthly income:

The average family income was 30,000 Naira in Akwa Ibom and Delta states and 50,000 Naira in Rivers state; families that earned above average constituted 32.6%, 31.7% and 54.3% in Akwa Ibom, Delta and Rivers states respectively. This difference in the family monthly income across the states was statistically significant ($X^2 = 216.0$, $p = 0.0001$) (Table 5).

Table 2. Survey informant (Respondent).

State	Akwa Ibom	Delta	Rivers	P-value/Fischers exact
Variable	**No (%)**	**No (%)**	**No (%)**	
Survey Informant				
Father	51 (5.1)	15 (1.5)	86 (7.6)	**47.15 (0.0001)***
Mother	920 (92.2)	964 (95.6)	1012 (88.9)	
Sister	8 (0.8)	7 (0.7)	7 (0.6)	
Grand Mother	7 (0.7)	10 (1.0)	12 (1.1)	
Aunty	10 (1.0)	7 (0.7)	14 (1.2)	
Others	2 (0.2)	5 (0.5)	7 (0.6)	

*Distribution is statistically significant ($p < 0.05$).

Table 3. Parents' educational level.

States	Akwa Ibom	Delta	Rivers	Chi-square/P value
Variable	**N (%)**	**N (%)**	**N (%)**	
Fathers' Education Level				
None	6 (0.6)	18 (1.8)	6 (0.5)	**210.6(0.0001) ***
Primary	96 (9.7)	49 (5.0)	20 (1.8)	
Secondary	492 (49.7)	559 (56.6)	405 (35.9)	
Tertiary	395 (39.9)	361 (36.6)	697 (61.8)	
Mothers' Education Level				**Fischer's exact/P-value**
None	3 (0.3)	26 (2.6)	4 (0.4)	**195.4(0.0001) ***
Primary	116 (11.7)	115 (11.5)	35 (3.1)	
Secondary	486 (48.9)	572 (57.1)	489 (43.2)	
Tertiary	389 (39.1)	288 (28.8)	605 (53.4)	

*Distribution is statistically significant ($p < 0.05$).

Table 4. Social class (Oyedele's social class classification).

State	Akwa Ibom	Delta	Rivers	Chi-square/P value
Variable	**No (%)**	**No (%)**	**No (%)**	
Social Class				
Upper Class	328 (32.9)	273 (27.2)	560 (49.4)	**175.6 (0.0001)***
Middle Class	617 (61.8)	617 (61.3)	548 (48.3)	
Low Class	53 (5.3)	116 (11.5)	26 (2.3)	

*Distribution is statistically significant ($p < 0.05$).

Malaria prevention techniques practiced in the three states

The techniques employed in the prevention of malaria the three states included; use of insecticide treated bed net (ITNs), insecticide spray, anti-malarial drugs, clearing of bushes and disposal of mosquito breeding cans and use of mosquito repellents as shown in Table 6. Use of ITN was practiced more in

Table 5. Family's monthly income.

State	Akwa Ibom	Delta	Rivers	Chi-square/P value
Variable	**N (%)**	**N (%)**	**N (%)**	
Family monthly income (Naira)				
<10,000	252 (25.5)	139 (14.2)	95 (9.5)	
10,000 - 50,000	413 (41.8)	528 (54.1)	364 (36.3)	**216.0 (0.0001)***
50,000 - 100,000	173 (17.5)	197 (20.2)	275 (27.4)	
>100,000	149 (15.1)	112 (11.5)	270 (26.9)	
Median family income	30,000 Naira	30,000 Naira	50,000 Naira	

*Distribution is statistically significant ($p < 0.05$). **one Naira equals 360 US dollars.

Table 6. Malaria prevention techniques practiced in the three states.

State	Akwa Ibom	Delta	Rivers	Fischer's exact
Prevention technique employed (Multiple response)	**N (%)**	**N (%)**	**N (%)**	
Use of ITNs	82 (8.2)	205 (20.3)	605 (53.2)	
Use insecticide spray	770 (77.2)	567 (56.3)	483 (42.4)	
None	200 (20.0)	359 (35.6)	182 (16.0)	
Use of drugs-antimalarial	286 (28.7)	134 (13.3)	133 (11.7)	**724.2 (0.0001)***
Others (Clearing of nearby bushes, disposal of mosquito breeding cans)	2 (0.2)	1 (0.1)	4 (0.4)	
Repellent	1 (0.1)	7 (0.7)	2 (0.2)	
Child slept under bed net last night				
No	931(93.3)	862 (85.5)	626 (55.0)	**500.6 (0.0001)***
Yes	67 (6.7)	146 (14.5)	512 (45.0)	
Have window nets				
No	439 (44.0)	319 (31.6)	168 (14.8)	**222.0 (0.0001)***
Yes	559 (56.0)	689 (68.4)	970 (85.2)	

ITNs—Insecticide treated bed nets.

Rivers state (53.2%) than in Delta (20.3%) and Akwa Ibom (8.2%) states, while use of insecticide spray was commoner in Akwa Ibom state (77.2%) than in Delta (56.3%) and Rivers (42.4%). The difference in the proportion of the different prevention techniques practiced among the states was statistically significant. (Fischer's exact −724.2, p-value = 0.0001)

Relationship between malaria Prevention techniques, social class and family income.

Tables 7-9 show the relationship between malaria prevention techniques, social class and the family income in Akwa Ibom, Delta and Rivers states respectively. In all the states, more people from the middle social class and those within

Table 7. Relationship between malaria prevention techniques, social class and family income in Akwa Ibom state.

Variable	Prevention techniques						
	ITNs	Insecticide sprays	Drugs	None	Repellent	Others	Fishers' Exact/P-value
Social status							
Upper class	18 (22.0)	266 (34.5)	83 (29.0)	101 (50.5)	1 (100.0)	1 (50.0)	
Middle class	53 (64.6)	471 (61.2)	182 (63.6)	91 (45.5)	0 (0.0)	1 (50.0)	**45.04 (0.0001)***
Low class	11 (13.4)	33 (4.3)	21 (7.3)	8 (4.0)	0 (0.0)	0 (0.0)	
Family income (Naira)**							
<10,000	18 (22.0)	192 (25.2)	79 (27.7)	15 (7.6)	0 (0.0)	1 (50.0)	
10,000 - 50,000	45 (54.9)	299 (39.3)	137 (48.1)	74 (37.4)	0 (0.0)	0 (0.0)	**79.73 (0.0001)***
50,000 - 100,000	9 (11.0)	146 (19.2)	34 (11.9)	49 (24.7)	0 (0.0)	0 (0.0)	
>100,000	10 (12.2)	124 (16.3)	35 (12.3)	60 (30.3)	1 (100.0)	1 (50.0)	

*Distribution is statistically significant ($p < 0.05$). **one Naira equals 360 US dollars.

Table 8. Relationship between prevention techniques, social status and family income in delta state.

Variable	Prevention techniques						
	ITNs	Insecticide sprays	Drugs	None	Repellent	Others	Fishers' Exact/P-value
Social status							
Upper class	26 (12.7)	191 (33.7)	31 (23.1)	98 (27.3)	2 (28.6)	0 (0.0)	
Middle class	138 (67.3)	334 (58.9)	83 (61.9)	218 (60.7)	5 (71.4)	1 (100.0)	**51.30 (0.0001)***
Low class	41 (20.0)	42 (7.4)	20 (14.9)	43 (12.0)	0 (0.0)	0 (0.0)	
Family income							
<10,000	34 (17.6)	62 (11.1)	41 (32.3)	52 (14.9)	2 (28.6)	0 (0.0)	
10,000 - 50,000	123 (63.7)	286 (51.3)	60 (47.2)	174 (50.0)	3 (42.9)	1 (100.0)	**70.94 (0.0001)***
50,000 - 100,000	22 (11.4)	124 (22.2)	18 (14.2)	89 (25.6)	2 (28.5)	0 (0.0)	
>100,000	14 (7.3)	86 (15.4)	8 (6.3)	33 (9.5)	0 (0.0)	0 (0.0)	

*Distribution is statistically significant ($p < 0.05$). **one Naira equals 360 US dollars.

Table 9. Relationship between Prevention technique, social status and family income in Rivers state.

Variable	Prevention techniques						
	ITNs	Insecticide spray	Drugs	None	Repellent	Others	Fishers' Exact/P-value
Social status							
Upper class	316 (52.2)	287 (59.4)	50 (37.6)	62 (34.1)	0 (0.0)	0 (0.0)	
Middle class	276 (45.6)	191 (39.5)	75 (56.4)	110 (60.4)	2 (100.0)	4 (100.0)	**61.41 (0.0001)***
Low class	13 (2.1)	5 (1.0)	8 (6.0)	10 (5.5)	0 (0.0)	0 (0.0)	
Family income (N)							
<10,000	44 (8.1)	19 (4.5)	24 (19.8)	21 (13.9)	0 (0.0)	0 (0.0)	
10,000 - 50,000	178 (32.8)	119 (27.9)	62 (51.2)	77 (51.0)	2 (100.0)	2 (50.0)	**106.5 (0.0001)***
50,000 - 100,000	167 (30.8)	135 (31.7)	23 (19.0)	26 (17.2)	0 (0.0)	2 (50.0)	
>100,000	153 (28.2)	153 (35.9)	12 (9.9)	27 (17.9)	0 (0.0)	0 (0.0)	

*Distribution is statistically significant ($p < 0.05$). **one Naira equals 360 US dollars.

the average monthly income practiced the different prevention methods compared to the lower and upper social classes. There was a statistically significant difference between the malaria prevention techniques practiced and the social class and family income in all the states (p value < 0.05).

4. Discussion

Malaria prevention and control is a key factor in reduction of infant and under-five morbidity and mortality, so assessment of preventive methods of malaria practiced in any community is a key first step to its control. This study showed that all three states practiced similar malaria preventive methods but with varying proportion and this includes the use of Insecticide treated bed nets (ITNs), insecticide spray, antimalarial drug use, mosquito repellants, clearing of nearby bushes and disposal of mosquito breeding cans.

Unlike in Rivers state where ITNs use (53.2%) was the commonest malaria preventive practice, the use of insecticide spray (a modified form of indoor residual spraying) was the common practice in Akwa Ibom state (77.2%) and Delta state (56.3%). According to the World Health Organization, the most powerful and widely applied system of control for malaria-carrying insects is the use of insecticides, mostly in the form of insecticide-treated bed nets (ITNs) and indoor residual spraying (IRS) [16]. This modified form of IRS that is commonly practiced among the people of Akwa Ibom and Delta states is usually short lived and requires repeated spraying as its effect wears off in less than 24 hours. This modified form of IRS was also practiced in 42.4% of residents in Rivers state. Therefore, in this area of stable malaria and malaria holoendemicity, it may not be an effective malaria control measure as its repeated use will be cost intensive. Its effectiveness can only be guaranteed in families that practice its regular use than where it is used irregularly, however, there is limited study in this form of insecticide use to show its effectiveness in malaria vector control.

Use of IRS was however not reported in any of the study sites despite its proven and well-established efficacy in malaria vector control [16]. IRS has been used widely in Asia, the Pacific and Latin America, and it is now being used in Africa. Wagman *et al.* [17] in an observational study in Mali, reported a 70% reduction in malaria cases following IRS use and a 70% increase in under-5 years old malaria incidence when its use was suspended. Akogbeto *et al.* [18] also reported a dramatic decrease in Malaria transmission after Large-Scale IRS in Benin republic. IRS involves coating the walls and other surfaces of a house with a residual insecticide and for several months, the insecticide will kill mosquitoes and other insects that come in contact with these surfaces as many malaria vectors are considered "endophilic"; that is, the mosquito vectors rest inside houses after taking a blood meal. Since mosquitoes are particularly susceptible to control through IRS, its introduction and use in swampy Niger Delta is advocated.

As noted earlier, use of insecticide treated bed nets (ITNs) is the common malaria preventive method in Rivers state, however, only 20.3% and 8.2% of

families in Delta and Akwa Ibom states respectively practiced this method. ITNs use is an approved, effective and recommended WHO malaria control and preventive method and its proper and consistent use have been shown to reduce malaria transmission by up to 90% and prevent up to 44% of all-cause mortality among under-five children [3] [19] [20] [21]. Asides being lower than that in Rivers state, ITNs use rate in Akwa Ibom and Delta states is lower than that from other Nigerian and African studies [22]-[28]. Similar reasons as given by Paul *et al.* [3] and other study [29] were also given for this low use rate. Programs aimed at increasing the awareness, free donation and distribution and use of ITNs must be promoted in these states. Though its use in Rivers state is high compared to that of other states, it is yet to attain the target observed in some African countries [22] [23] [24] [25] [26] and therefore its ownership and use must also be promoted in Rivers state.

Use of antimalarial prophylaxis is an uncommon practice in children in Nigeria though it's a malaria preventive method recommended by WHO in pregnant women and infants in medium and high malaria transmission areas [30]. It is however practiced among pregnant women, children with sickle cell anaemia (SCA), non-immune visitors to endemic areas and children who have undergone splenectomy. Asides the huge cost implication of a mass antimalarial chemoprophylaxis in infants and children, it is also thought that the acquired immunity that makes severe malaria uncommon in older children may not be attained, hence it's not a common practice. Use of antimalarial drugs is low in this study among the three states and probably represents children with SCA which is quite common in Nigeria.

Other less common methods with lower community malaria prevention effectiveness practiced in less than 1% in the different states include clearing of bushes, emptying and disposal of containers where mosquitoes breed and use of mosquito repellent creams. Clearing of mosquito breeding places may clear the larval stages of mosquito but is presently a difficult task especially because there is irregular availability of portable water in many homes leading to prevalent dirty and stagnant gutters in many areas. It is also difficult to guarantee the consistent application of mosquito repellent creams by all members of a household or community.

In 16% - 35.6% of the participants across the three states, no malaria prevention method was practiced and this cuts across all the social and economic classes. This finding emphasizes the need for creating more awareness on malaria preventive practices to all and sundry in the Niger Delta states. In this study, 56% - 85.2% of the participants in the three states had window nets installed in their homes, however, the quality and physical status of these nets cannot be guaranteed as they are not maintained in many homes and so may be a poor vector preventive measure.

More proportion of people in the middle class and those whose monthly income was within the average median income practiced the different methods of

malaria prevention in the three states, while a lower proportion of people in the lower and upper class practiced any of the methods. High patronage of public hospitals by the middle class where education on ITNs use and its free distribution is done may have contributed to this. Though no social class is exempt from malaria in endemic areas, people from high social class live in cleaner areas of the city, have good protective window and door nets and are likely to have better health seeking behavior compared to those from the lower social class. It is therefore not surprising to have low rates of the malaria prevention methods among them. Lack of awareness and poverty are contributory factors to the low malaria prevention practices among the lower social class and those with low average monthly income. Contributory to this may also be due to the fact that since distribution of these nets mostly takes place in public hospitals which is often less patronised by individuals from the lower class who prefer patronage of quacks due to the huge costs in out of pocket expenditure on health bills they may not own one. This finding is an important one but is worrisome in that those who need these preventive practices the most, practice them the least and hence dying more from malaria. Public health education and refocusing of distribution centres and strategy by using schools, markets and other community gatherings may change this dynamic [3].

5. Conclusions

In conclusion, this study has shown that the practice of the two common and effective malaria preventive measures; IRS and ITNs use is low in South-South Nigeria with IRS not being practiced at all. Also, the low practice of ITNs and modified IRS among these states is worse among the lower social class who need it the most.

Introduction of IRS as a method of malaria vector control is advocated in South-South Nigeria. Public health education with emphasis on ITNs ownership and use and refocusing of distribution strategy by using schools, markets and other community gatherings will increase its availability and use among all especially among those who need it the most.

Author's Contribution

Author 1 wrote the first draft, Author 7 carried out the statistical analysis and results, Authors' 8 and 9 reviewed the first draft. All authors read and approved the final draft and are part of the Niger Delta Development Company (NDDC) Professorial Chair on Malaria Elimination & Phytomedicine Research, University of Port Harcourt that collected the data.

Limitation of the Study

Answers provided by the informants in this study were based on recall, and this may affect the validity of the responses.

Conflicts of Interest

There is no conflict of interest.

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The Effect of YiQiFuMai on Ischemic Heart Failure by Improve Myocardial Microcirculation and Increase eNOS and VEGF Expression

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Abstract

Objective: To assess the effects of traditional Chinese medicine YiQiFuMai on cardiac function during the progression of ischemic heart failure. **Methods:** Rabbits were divided into sham, heart failure, and YiQiFuMai groups. The ischemic heart failure model was established in New Zealand white rabbits, which were intraperitoneally injected with YiQiFuMai injection and 0.9% sodium chloride after the operation. After six weeks, cardiac function was examined by ultrasound; serum BNP levels were measured by ELISA; p-AKT, eNOS, ICAM-1 and VEGF levels were evaluated by real-time PCR and Western-Blot; pathological changes of the myocardial tissue were observed by H&E staining; CD31 expression in tissue samples was analyzed by immunohistochemistry. The ultrastructure and microcirculation of myocardial tissue specimens from the three groups were assessed by transmission electron microscopy. **Results:** YiQiFuMai decreased serum BNP levels, and increased LVEF and reduced LVEDD at 6 weeks postoperatively. In addition, YiQiFuMai can improve myocardial damage and microcirculation structure, as assessed by histology and transmission electron microscope. At the molecular level, treatment with YiQiFuMai resulted in increased eNOS, VEGF and p-AKT levels but reduced ICAM-1 amounts compared with the heart failure group. **Conclusion:** Ischemic heart failure damages the microvascular structure and functions of the myocardium. Treatment with YiQiFuMai potentially ameliorates microcirculatory damage and alleviates cardiac failure by improving endothelial function and angiogenesis, and inhibiting inflammatory cell adhesion.

Keywords

Endothelial Nitric Oxide Synthase (eNOS), Endothelial Cells, PI3K/AKT/eNOS Pathway, Microcirculation, Heart Failure, YiQiFuMai

1. Introduction

After acute myocardial infarction, myocardial fibrosis and cardiac dilatation occur gradually in the infarcted myocardium, eventually leading to heart failure [1]. Coronary ischemia causes hemodynamic disorder, excessive neuroendocrine activation and abnormalities in energy metabolism, which aggravate ventricular remodeling and cardiac dysfunction [2].

It has been highlighted that microcirculatory dysfunction plays a pivotal role in ischemic heart failure [3]. eNOS is required for producing NO, an important vasodilator substance that is indispensable to normal cardiac function. Its release is affected by a series of signaling pathways during endothelial injury, leading to coronary microvascular damage [4]. Endothelial damage is associated with excessive oxidative stress and inflammatory response, as well as upregulation of adhesion molecules such as ICAM-1 and VCAM-1, resulting in platelet aggregation and adhesion that affect microcirculation [5].

Microvascular angiogenesis is crucial to survival and functional recovery after ischemic heart disease. Angiogenesis is triggered by angiogenic factors binding to endothelial cell receptors, and VEGF is the major factor contributing to angiogenesis [6]. VEGF plays an important role in angiogenesis through three structurally related VEGF receptor tyrosine kinases, including VEGFR1, VEGFR2 and VEGFR3 [7]. VEGFR2, a major VEFG receptor in endothelial cells, is indispensable for the differentiation, proliferation and migration of endothelial cells, as well as angiogenesis. Additionally, VEGF can increase vascular permeability to facilitate angiogenesis by binding to VEGFR2. VEGF is critically involved in the maturation of newly formed blood vessels in ischemic myocardium, providing long-term blood supply for cardiomyocytes and limiting ventricular remodeling after myocardial infarction [8].

A variety of drugs are currently used to improve cardiac microcirculation. Yiqifumai Injection (YQFM), is composed of *Panax ginseng*, *Ophiopogon japonicas*, and *Schisandra chinensis* (Ginseng). Li, F., *et al.* found schisandrin pretreatment inhibited cell apoptosis, as evidenced by inhibiting activation of caspase-3 and increasing the Bcl-2/Bax ratio. Meanwhile, the vascular endothelium protective property of schisandrin might be beneficial for the treatment of cardiovascular disease [9]. A study by China demonstrated that YQFM could ameliorate myocardial apoptosis via positive regulation of AMPK, PI3 K/Akt and negative regulation of MAPKs signaling pathways. Besides, ginsenoside Rd might partially adjust omentin-dependent protective effect of YQFM [10]. In addition, they also found that YQFM markedly attenuated mitochondrial dysfunction through improving mitochondrial morphology, increasing mitochondria membrane potential (1 μm), mitochondrial ROS generation and expression of Mitofusin-2 (Mfn2), meanwhile, decreasing phosphorylation of dynamin-related protein 1 (p-Drp1) [11]. In this study, we assessed the beneficial effects of YiQiFuMai on myocardial microcirculation in rabbits during ischemic heart failure. We showed that treatment with YiQiFuMai resulted in increased levels of the protective molecules eNOS and VEGF, while downregulating ICAM-1.

2. Materials and Methods

The schedule for animal experiments is as shown in Figure 1.

Experimental animals

A total of 30 healthy New Zealand male rabbits aged 3 months (approximately 2.5 ± 0.3 kg) were used in this study. The animals were provided by the Experimental Animal Center of Hebei Medical University, and randomly divided into the sham operation, heart failure, YiQiFuMai groups, with 10 animals per group. After successful establishment of the heart failure model, YiQiFuMai injection (lyophilized) (Tianjin Zhijiao Pharmaceutical Co., Ltd. of Tasly Holding Group) at a concentration of 270 mg/mL in normal saline was intraperitoneally administered at 2 mL/(kg·d) for 4 weeks in the YiQiFuMai group. The sham operation and heart failure groups were intraperitoneally administered normal saline at 2 mL/(kg·d) for 4 weeks.

Establishment of the experimental animal model

The ischemic heart failure model was established by ligating the anterior descending coronary artery of rabbits [12]. After weighing, sodium pentobarbital anesthesia was performed via the ear vein. Preoperative ECG was recorded using an electrocardiograph. Skin and subcutaneous tissues were cut along the left margin of the sternum. The pericardium was cut off to expose the anterior descending artery of the heart. The anterior descending branch was ligated using No.0 lines, 2 - 3 mm below the junction between the pulmonary conus and the left atrial appendage. Then, the chest was sutured. In the sham operation group, only the chest was open, and the anterior descending coronary artery was not ligated. After modeling, the apex and the left ventricular anterior wall did not change in color, myocardial motion did not decrease, and precordial ST segment did not raise. After the operation, all rabbits were intramuscularly injected 800,000 units of penicillin daily for a total of 3 days. Cardiac ultrasonography was performed 2 weeks after modeling, and the ischemic heart failure model was considered to be successfully established with LVEF below 50%.

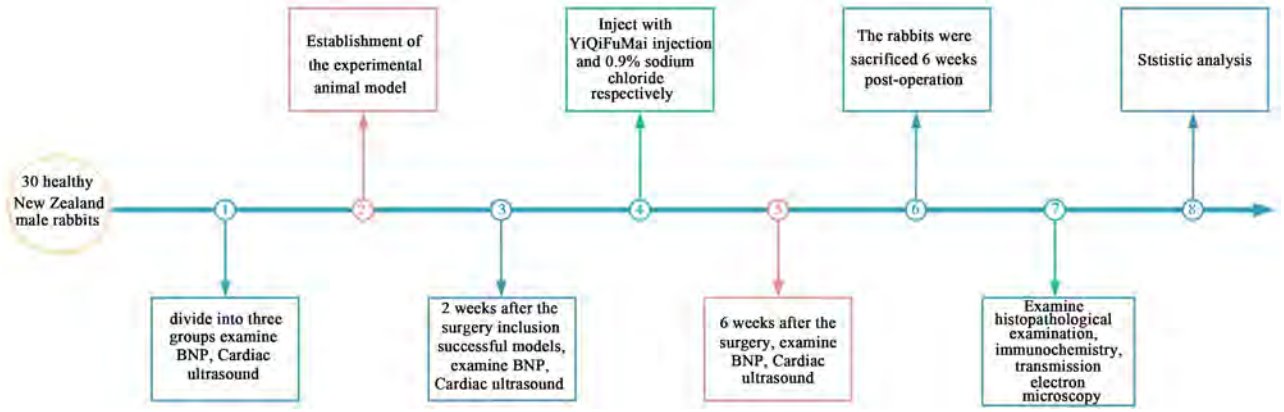


Figure 1. The schedule for animal experiments.

Cardiac function test by cardiac ultrasonography

Cardiac function was tested by the same cardiac sonographer using a cardiac echocardiography device (ALOKA) 2 weeks before the operation and 6 weeks postoperatively. The rabbits were fixed on a table to remove chest fur to expose the skin. The ultrasonic probe was replaced with a cardiac ultrasound probe at a frequency of 5.0 MHZ. Ventricular wall motion was observed from the long-axis view of the sternum left ventricle. The mitral septal angle horizontally and the curve of left ventricular posterior wall were assessed by M-mode ultrasound to measure LVEF and LVEDD. Mean values from three measurements were calculated.

Detection of serum BNP levels by ELISA

A total of 4 ml of blood was collected via the middle auricular artery from each animal before the operation, and 2 and 6 weeks post-operation, respectively, without anti-coagulation. Blood samples were placed at room temperature for 2 h, then centrifuged at 3000 rpm for 15 min for serum preparation. Operations were based on the ELISA kit (Shanghai Enzyme Biotechnology Co., Ltd.) instructions. A regression formula was generated according to the concentrations of standards and the corresponding absorbance values. BNP levels were derived based on the above formula.

Histopathological examination

Rabbits were sacrificed 6 weeks after the operation to harvest myocardial tissues in the infarcted border areas. Gradient dehydration was performed with ethanol after tissue fixation with paraformaldehyde. Dehydrated tissue samples were paraffin embedded and sliced transversely into 5μm sections using a slicer. Before staining, the tissue sections were dewaxed with xylene. Staining with H & E followed routine procedures. Finally, observation was performed on an AHB-2-HL optical microscope (Olympus, Japan).

Immunochemistry

After slicing the paraffin blocks as described above, the sections were dewaxed and hydrated. Then, antigen retrieval, incubation with 3% H_2O_2 in deionized water, and sealing with goat serum were performed, followed by incubation with mouse anti-rabbit CD31 primary antibodies (1:50) (BIOSS/bs-0195R) at 4˚C overnight. Next, goat anti-mouse IgG II antibodies (Santa/Sc-2005) and streptavidin-horseradish peroxidase (S-A/HRP) were added dropwise for incubation at room temperature. Finally, DAB staining, conventional counterstaining, dehydration and film sealing with neutral gum were performed. The cytoplasm of positive vascular endothelial cells was stained brown.

Assessment of myocardial tissue ultrastructure by transmission electron microscopy

Six weeks post-operation, the whole heart was extracted by opening the chest once again, and rinsed thoroughly with cooled saline. Myocardial tissues of the left ventricular anterior wall were harvested along the direction of myocardial fibers in the infarcted border areas at a size of $1 \times 1 \times 3$ mm^3. The harvested myocardial tissue samples were immediately placed in 4% glutaraldehyde for

overnight fixation, followed by rinsing, fixation, dehydration with gradient acetone, immersion, embedding and ultra-thin slicing after polymerization (50 nm). Tissue slices were successively stained with uranyl acetate for 30 - 45 min and lead citrate for 5 - 30 min. Observation was performed under a Hitachi H-7500 transmission electron microscope (Japan Hitachi, Ltd.).

Real-time qPCR

The rabbits were sacrificed 6 weeks post-operation. The myocardial tissue (50 mg) from the infarcted area was homogenized for total RNA extraction. Reverse transcription was performed according to the reverse transcription kit's instructions. Primer sequences are shown in Table 1. The obtained cDNA was subjected to real-time fluorescent quantitative PCR. β-actin was used as an internal reference, and the $2^{-\Delta\Delta Ct}$ method was employed for analysis (ΔCt = Ct value of the target gene—Ct value of β-actin; $\Delta\Delta Ct$ = ΔCt value of the target gene—ΔCt value of β-actin).

Immunoblotting

The rabbits were sacrificed 6 weeks post-operation. After weighing, myocardial tissue samples from the infarcted border areas were homogenized for protein extraction. Total protein was extracted with RIPA buffer supplemented with protease and phosphatase inhibitors. The samples were then subjected to polyacrylamide gel electrophoresis and transferred onto PVDF membranes, which were immersed in Western blot blocking solution. After incubation with primary antibodies (1:1000) raised against p-Akt (BIOSS/bs-0876R), eNOS (Abcam/ab5589), ICAM-1 (BIOSS/bs-4617R), VEGF (Abcam/ab1316) and β-actin (Abcam ab8226) at 4˚C overnight, respectively, the membranes were washed and subjected to incubation with secondary antibodies (1:5000). ECL solution was used for development. The protein levels of eNOS, ICAM-1, p-AKT and VEGF were then quantified.

Statistical analysis

Data were analyzed with the SPSS 21.0 software. Continuous data with normal distribution were represented by mean ± standard deviation (Mean ± SD) and compared by one-way ANOVA. Pairwise comparisons of multiple samples were performed by the SNK-q test. $P < 0.05$ was considered statistically significant.

Table 1. Primers for real-time PCR.

Primer	Sequences (5' - 3')
eNOS-Forward	ACCTTCGTTCAGCCATCACAGT
eNOS-Reverse	GACACCTCCAGGACCAACTCAG
ICAM-1-Forward	GACTGCCTGGGAAACTGGACTT
ICAM-1-Reverse	GCCGCCACCACGATGATGAT
VEGF-Forward	TTCATGGAAGTCTACCGGCG
VEGF-Reverse	TGACGTTGAACTCCTCGGTG
β-action-Forward	AGATCGTGCGGGACATCAAG
β-action-Reverse	CAGGAAGGAGGGCTGGAAGA

3. Results

YiQiFuMai improve heart failure in the rabbit model

A total of 30 New Zealand white rabbits were subjected to modeling, and 24 survived. In each group, 8 rabbits survived and 2 died. A total of 6 rabbits died. Among them, 2 animals died of pneumothorax, 2 of ventricular fibrillation, 1 of excessive anesthesia, and 1 of excessive bleeding due to puncture of the heart.

At 6 weeks after operation, the heart failure, YiQiFuMai groups showed markedly increased LVEF in comparison with the values at 2 postoperative weeks ($P < 0.05$), while there were no significant differences in the sham group for LVEF and LVEDD at these times (Table 1). There were no significant differences for LVEDD in the heart failure group at these time points. LVEDD was significantly decreased in the YiQiFuMai groups. Compared with the heart failure group, the YiQiFuMai showed significantly increased LVEF, and markedly reduced LVEDD (Table 1).

There were no statistically significant differences in LVEF and LVEDD among groups before operation ($P > 0.05$). Two weeks after the surgical operation, we found that LVEF values in the heart failure, YiQiFuMai groups were significantly increased compared with preoperative values, while LVEDD values in these three groups were significantly decreased after modeling (Table 2). Besides, LVEF and LVEDD significantly differed, with increased LVEF and reduced LVEDD in animals with heart failure (heart failure, YiQiFuMai groups) compared with the sham group (Table 2).

We analyzed the LVEDD and LVEF of each animal before operation, 2 weeks after operation, and 6 weeks after operation, as shown in the box chart (Figure 2).

Serum BNP concentrations are reduced in the YiQiFuMai group

Next, serum levels of BNP were assessed by ELISA. We found that there were no statistically significant differences among groups before operation. Two weeks after the operation, BNP concentrations in the sham operation group were not significantly different from those before modeling, while the heart failure, YiQiFuMai groups showed significantly increased serum BNP amounts compared with preoperative values. Compared with the sham operation group, the heart failure, YiQiFuMai groups showed significantly increased serum BNP levels at 2 weeks postoperatively (Figure 3).

Table 2. LVEF and LVEDD values preoperatively, and at 2 and 6 weeks after operation.

	Group	Sham Group	Heart failure group	YiQiFuMai group
LVEF (%)	Preoperative	69.2 ± 5.8	65.3 ± 4.1	66.0 ± 3.9
	2 weeks	66.1 ± 7.6*	43.7 ± 5.1	41.0 ± 6.3
	6 weeks	67.3 ± 7.0*	52.4 ± 7.4	59.7 ± 7.4*
LVEDD (mm)	Preoperative	12.5 ± 1.4	12.5 ± 1.7	12.5 ± 1.6
	2 weeks	12.3 ± 1.1*	14.0 ± 1.2	13.5 ± 0.8
	6 weeks	11.3 ± 1.9*	13.5 ± 2.2	11.7 ± 1.3*

*$P < 0.05$ vs heart failure group.

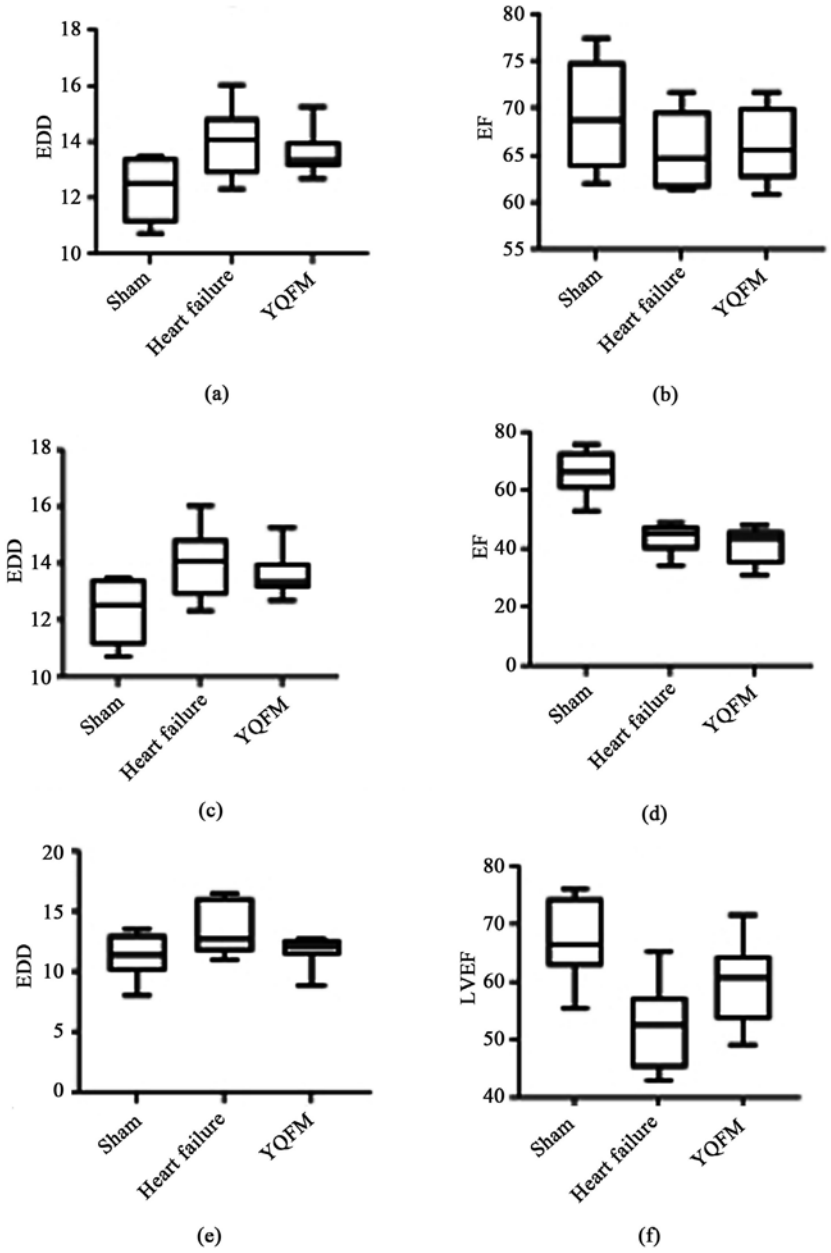


Figure 2. LVEF and LVEDD values preoperatively, and at 2 and 6 weeks after operation. (a) LVEDD preoperation (Sham group, Heart failure group, YQFM group); (b) LVEF preoperation (Sham group, Heart failure group, YQFM group); (c) LVEDD 2 weeks (Sham group, Heart failure group, YQFM group); (d) LVEF 2 weeks (Sham group, Heart failure group, YQFM group); (e) LVEDD 6 weeks (Sham group, Heart failure group, YQFM group); (f) LVEF 6 weeks (Sham group, Heart failure group, YQFM group).

At 6 weeks post-operation, the sham operation and heart failure groups showed no significant differences in BNP compared with values at 2 postoperative weeks, In the YiQiFuMai group, serum BNP at 6 postoperative weeks was significantly lower than that of the same group at 2 weeks post-operation. Compared with the heart failure group, there was no statistically significant difference in the YiQiFuMai groups in serum BNP levels (**Figure 3**).

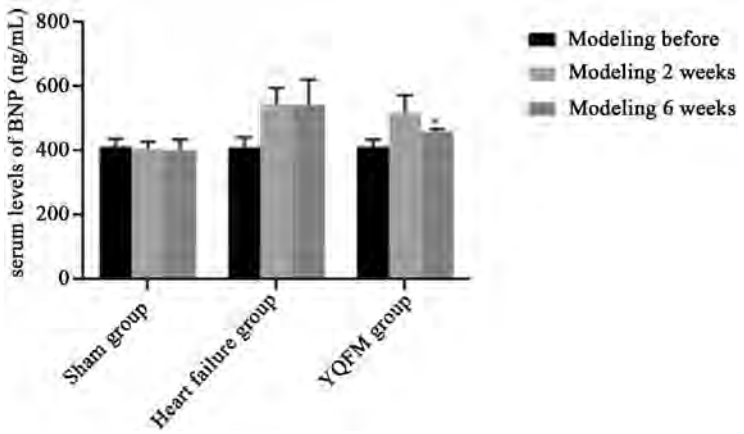


Figure 3. Serum BNP levels. Serum BNP was measured by ELISA in the sham, heart failure and YiQiFuMai groups preoperatively, and 2 and 6 weeks after the operation. *P < 0.05 vs heart failure group at the same time point.

Pathological damage in the myocardial tissue is reduced by YiQiFuMai

In the heart failure group, H&E staining showed disordered arrangement of myocardial fibers, lysed cardiomyocyte nuclei, and red-stained cytoplasm. Myocardial edema, cell degeneration in the infarcted areas, and peripheral infiltration of inflammatory cells were also observed. Compared with the heart failure group, YiQiFuMai groups had reduced disease severity (**Figure 4**).

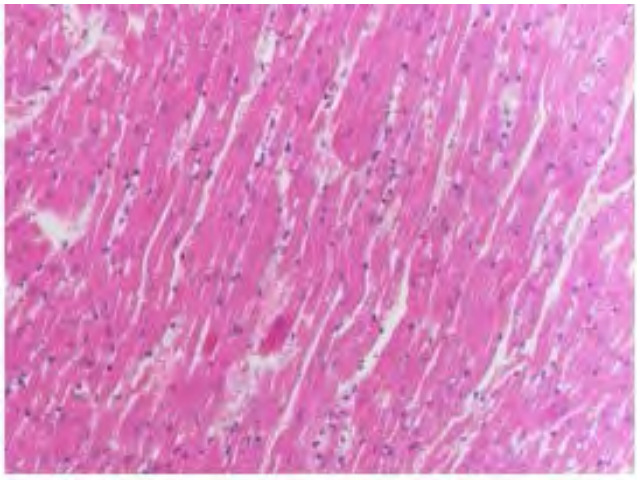
Treatment with YiQiFuMai increases capillary density after heart failure

Brown capillary endothelial cells stained by CD31 were visible in the myocardial tissues of each group six weeks post-operation. Compared with the heart failure group, theYiQiFuMai groups showed elevated capillary densities (**Figure 5**).

Improved ultrastructure and microcirculation of the myocardium by YiQiFuMai

Transmission electron microscopy showed that the sham-operated group had orderly arranged myofilaments, with abundant mitochondria arranged along myocardial fibers (**Figure 6**). Then, the myocardial microvascular ultrastructure was also assessed (**Figure 7**). The sham group had smooth myocardial microvascular lumen and intact basal lamina (**Figure 7**). In addition, there were pinocytotic vesicles in the cytoplasm and tight connections between endothelial cells in the sham group (**Figure 7**). In the heart failure group, the myocardial tissue ultrastructure showed broken myofilaments, disordered arrangement of the mitochondria, disappearing ridge, and vacuolar degeneration (**Figure 6**). For myocardial microvascular structure, the heart failure group had irregular microvascular lumen, broken basal lamina, and reduced number of pinocytotic vesicles (**Figure 6**). In the YiQiFuMai groups, the above abnormalities were improved (**Figure 6** and **Figure 7**).

The mRNA expression levels of eNOS and VEGF are increased while ICAM-1 gene expression is decreased in infarcted areas of myocardial tissues from the YiQiFuMai groups



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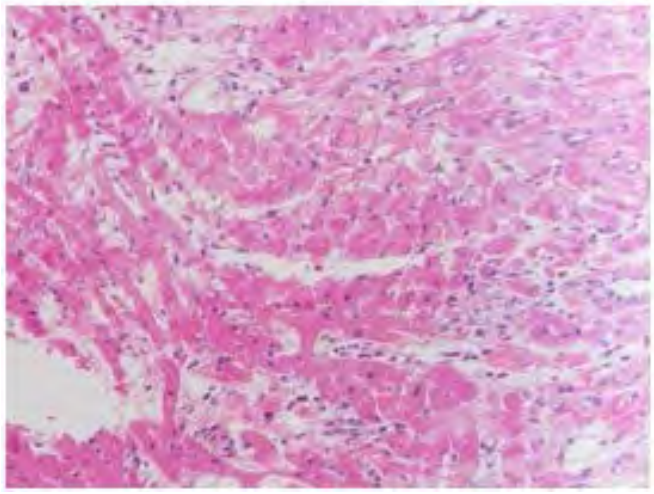

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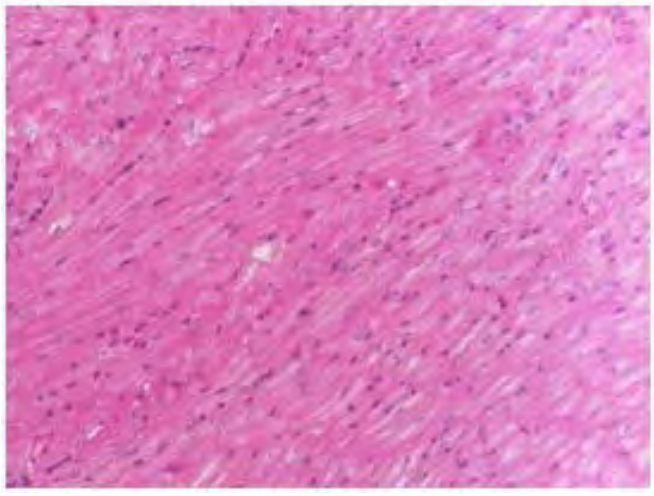
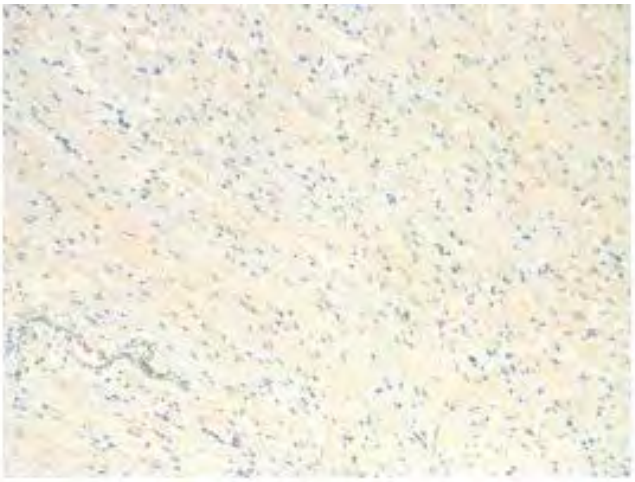
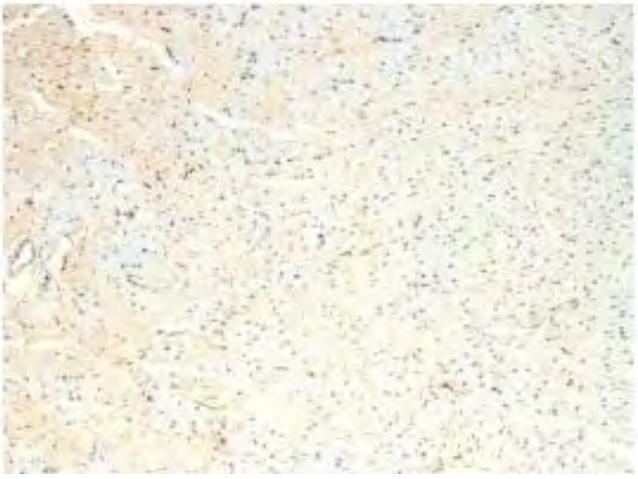


(c)

Figure 4. YiQiFuMai improve myocardial pathology. H&E staining of myocardial tissue samples from the sham (a), heart failure (b) and YiQiFuMai (c) groups (10 × 20) six weeks after the operation.



(a)



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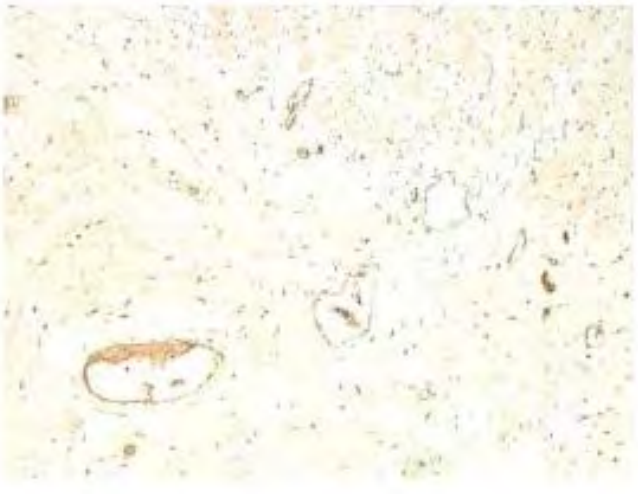
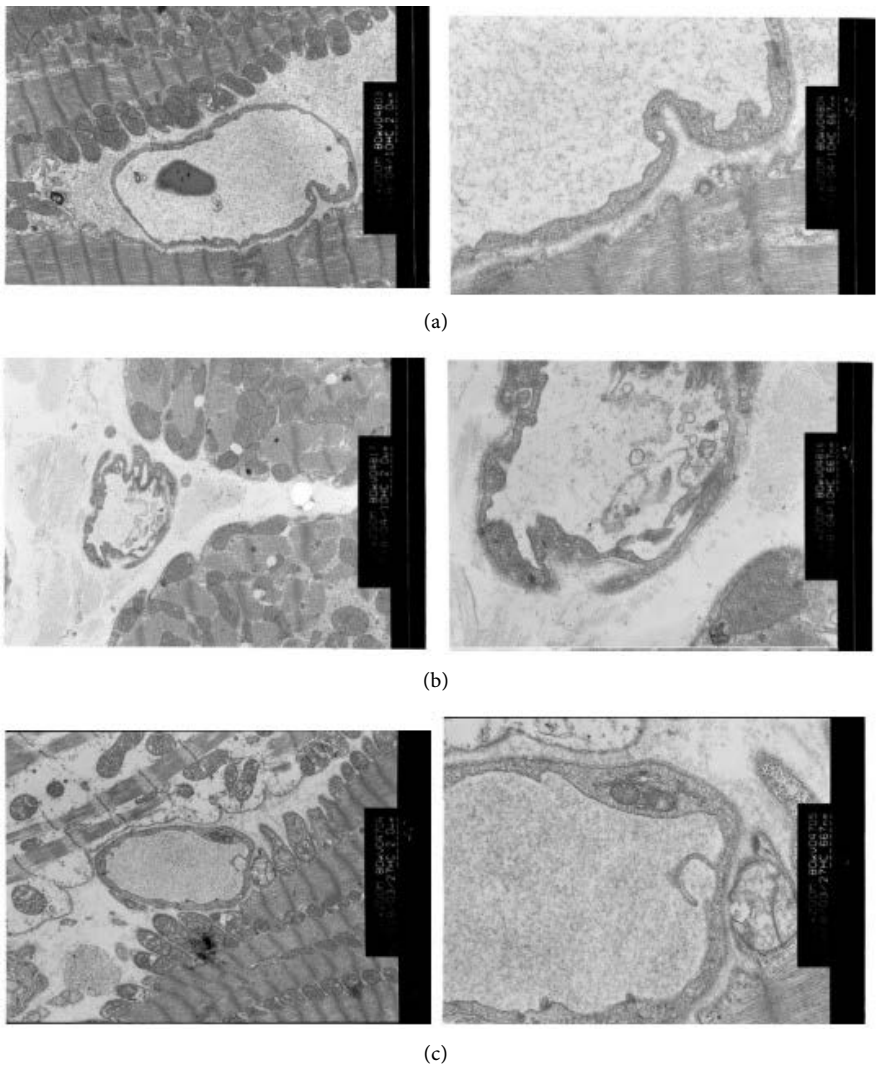


(c)

Figure 5. YiQiFuMai increase the amounts of capillary endothelial cells. Immunohistochemical staining of CD31 in myocardial tissue samples from the sham (a), heart failure (b) and YiQiFuMai (c) groups (10 × 20) six weeks after the operation.



(a)

(b)

(c)

Figure 6. YiQiFuMai improve myocardial structure. Representative transmission electron micrographs of myocardial tissue samples from the sham (a), heart failure (b) and YiQiFuMai (c) groups six weeks after the operation (left: 5000×, right: 15,000×).

To further investigate the molecular mechanisms underlying the observed phenotype, the mRNA expression levels of endothelial nitric oxide synthase (eNOS), ICAM-1, and VEGF were analyzed in infarcted areas of myocardial tissues. The heart failure group showed a trend towards decreased eNOS and increased ICAM-1 mRNA levels compared with the sham group (**Figure 8**). VEGF expression was similar between the two groups (**Figure 8**). Compared with the heart failure group, the YiQiFuMai group had significantly elevated eNOS levels (**Figure 8**). Furthermore, decreased ICAM-1 gene expression and increased VEGF mRNA amounts were detected in YiQiFuMai group compared with the heart failure group (**Figure 8**).

AKT activation and eNOS and VEFG protein levels are increased, while ICAM-1 protein amounts are reduced in the infarcted areas of myocardial tissues from the YiQiFuMai group

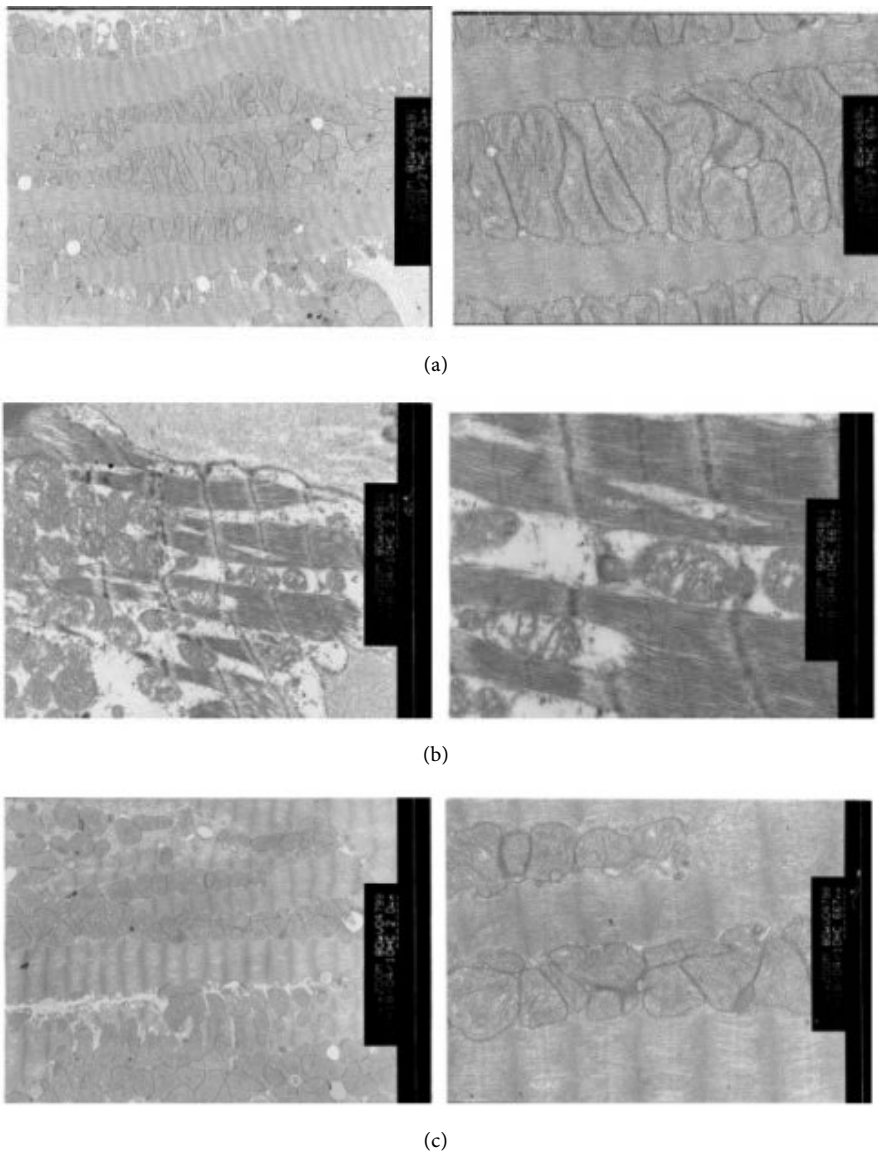


Figure 7. YiQiFuMai improve myocardial microvascular structure. Representative transmission electron micrographs of myocardial microvascular structure in the sham (a), heart failure (b) and YiQiFuMai (c) groups six weeks after the operation (left: 5000×, right: 15,000×).

Consistent with mRNA data, the heart failure group had reduced protein levels of eNOS, increased ICAM-1 protein amounts, and similar protein levels of VEGF compared with the sham group. eNOS is considered a downstream target of the PI3K/AKT signaling pathway. We found that p-AKT protein levels were decreased in the heart failure group suggesting reduced activation of the AKT pathway after heart failure (**Figure 9**).

The YiQiFuMai group showed decreased ICAM-1 protein amounts and increased p-AKT and VEGF levels compared with the heart failure group. For eNOS protein levels, treatment with t YiQiFuMai had no upregulation compared with the heart failure group (**Figure 9**).

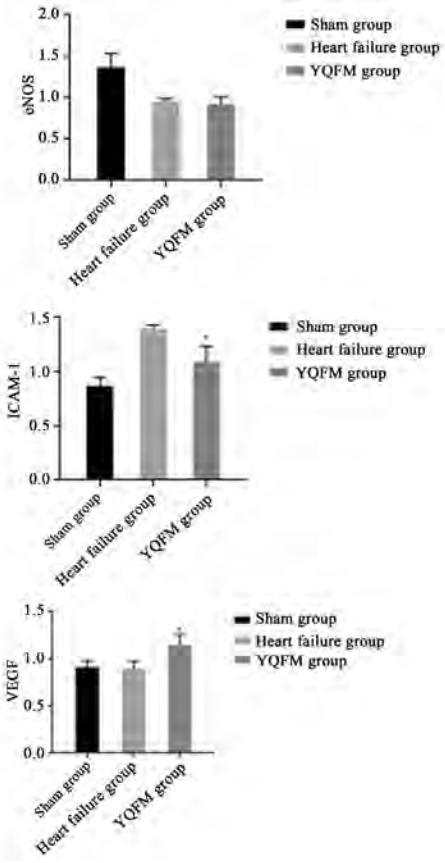


Figure 8. Increased eNOS and VEGF mRNA levels, and decreased ICAM-1 gene expression in response to YiQiFuMai treatments. Real-time qPCR analysis of genes encoding eNOS, ICAM-1 and VEGF six weeks after the operation. *P < 0.05 vs heart failure group.

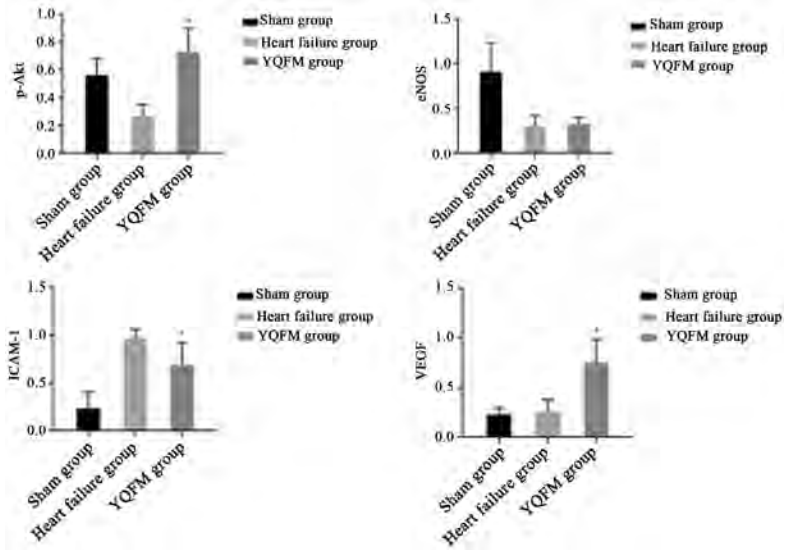


Figure 9. Increased AKT activation and VEGF protein levels, and decreased ICAM-1 protein amounts in response to YiQiFuMai treatments. Immunoblotting analysis of the pAKT, eNOS, ICAM-1, and VEGF proteins in the sham, heart failure, and YiQiFuMai groups 6 weeks after the operation. β-actin was used as a loading control. *P < 0.05 vs heart failure group.

4. Discussion

It was suggested that microvascular dysfunction is one of the major processes in the development of ischemic heart failure [12]. The endothelium of coronary vessels could regulate coronary blood flow in epicardial coronary and intramyocardial microcirculations [13]. Coronary blood flow increase partly depends on the regulation of endothelial function. It can be speculated that microvascular dysfunction may lead to recurrent episodes of myocardial ischemia and small infarctions that eventually result in heart failure.

Endothelial dysfunction occurs when myocardial NO generation decreases due to eNOS uncoupling and excessive peroxide production followed by nitrogen oxide activation [14]. Recent studies suggested that eNOS is a potential target of PI3K/AKT signaling. The activation of AKT leads to serine 1177 phosphorylation of eNOS, which can upregulate the expression of eNOS. In addition, activation of the PI3K/AKT/eNOS pathway can inhibit apoptosis in vascular endothelial cells [15]. AKT plays an important role in reducing cardiomyocyte apoptosis and myocardial protection. Activation of the PI3K/AKT pathway and upregulation of eNOS expression suppresses pro-apoptotic factors and activates the anti-apoptotic pathway to maintain the stability of the outer mitochondrial membrane and improve mitochondrial energy metabolism, thus inhibiting cell apoptosis and protecting the myocardium [16]. In this study, we showed that YiQiFuMai could provide protection to endothelial cells by reducing endothelial cell apoptosis and endothelial dysfunction. These events would eventually improve myocardial microcirculation and cardiac function.

Left ventricular dysfunction and heart failure are closely related to increased proinflammatory cytokine and ICAM-1 levels [17]. ICAM-1 is an immunoglobulin-like adhesion molecule, which is expressed during inflammatory stimulation of endothelial blood vessels. ICAM-1 induces inflammatory reactions by mediating white blood cell migration to the inflammatory site and adhesion to endothelial blood vessels [18]. Recent studies suggested that ICAM-1 expression in the heart and coronary endothelium can cause damage to the endothelium of coronary arteries, thus worsening the disorder of coronary microcirculation [19]. The present study showed that ICAM-1 expression was increased significantly in heart failure, and YiQiFuMai treatments downregulated ICAM-1 in vascular endothelial cells, which may reduce the damage to the vascular endothelium, thereby protecting cardiac microcirculation.

Angiogenesis is essential for myocardial cell survival and ventricular function recovery after myocardial infarction. A study by Chen *et al.* revealed that excessive expression of VEGF can inhibit cardiomyocyte apoptosis [20]. Ischemic reactions can induce the expression and activation of VEGF, causing microvascular dilation and neovascularization [21]. A study by Shen *et al.* showed that VEGF is excessively expressed in hypertrophic cardiomyocytes induced by pressure overload, which stimulates cardiac microvascular endothelial cells and induces neovascularization. This process may be mediated by activated

PI3K/AKT/ Hif-1α pathway [22]. Zou *et al.* also demonstrated that upregulation of VEGF in the rat model of acute myocardial infarction stimulates ROS production, which in turn induces oxidative stress in the endoplasmic reticulum, as well as autophagy, thereby promoting cardiac neovascularization [23]. This study revealed that VEGF protein amounts were increased after treatment with YiQiFuMai. CD31 expression was also increased, suggesting that both treatments could potentially improve microcirculation by promoting neovascularization.

This study showed microvascular structure damage and dysfunction in ischemic heart failure. After application of the above drugs, the ejection fraction was improved, and BNP levels were decreased. Moreover, cardiac and microvascular structures were also improved as assessed by pathology and electron microscopy. Improving microcirculation to reverse cardiac dysfunction has been recently recognized as a promising clinical intervention in heart diseases. VEGF expression was also increased by YiQiFuMai. These events together are thought to protect microcirculation and improve ventricular remodeling. Further studies using PI3K or Akt inhibitors may directly reveal the relationship between eNOS and the PI3k/AKT pathway. In addition, the optimal drug concentrations required to treat the disease should be further investigated. Overall, this study observed microcirculatory alterations during the development of ischemic heart failure, which is associated with microvascular endothelial dysfunction. YiQiFuMai improved myocardial pathology and microcirculation, potentially via induced PI3K/AKT/eNOS pathway and VEGF upregulation.

Conflicts of Interest

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

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