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OPEN ACCESS THE EFFECT OF SUPPORTIVE COUNSELING WITH ACUPRESSURE ON NAUSEA AND VOMITING IN PREGNANT WOMEN REFERRING TO HEALTH CENTERS IN HAMADAN, IRAN: A RANDOMIZED DOUBLE BLIND CONTROLLED TRIAL STUDY

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#### ABSTRACT

Objective: To measure the effect of supportive counseling and acupressure on P6 point on nausea and vomiting.

Methodology: This study is a randomized clinical trial with two groups. And has been done in the health clinic centers of Hamadan, Iran, in 2020. A total of 60 pregnant women were randomly divided into two groups. Group 1 received three face-to-face support counseling sessions by a midwife, one session every week, and continuous follow-ups by phone or online, using a 24-hour P6 acupressure wristband for fourteen days, and group 2 had the same number of sessions with using a placebo wristband. The severity of nausea and vomiting was assessed based on PUQE-24 questionnaire item for 17 days.

Results: The mean age of pregnant women in two groups were 28.10 (5.13) and 27.93 (14.71) respectively. Nausea exacerbating variables and level of spouse information about pregnancy, appropriate verbal, sexual and emotional communication with spouse, level of domestic violence, level of information about nausea reduction methods in both groups were homogeneous (p>0.05). There was a decreasing trend in the mean scores of questions 1(duration of nausea or stomach problems), 2 (number of vomiting times during the day) and 3 (number of retching per day) after the intervention (p < 0.001). But the trend of decreasing scores in the first group was more than the second group. Although there was no significant difference (p>0.05).

Conclusion: Based on the findings, acupressure on P6 combined with supportive counseling is indicated to minimize vomiting and nausea during pregnancy.

Keywords: Counseling; Nausea; Vomiting; Acupressure; Pregnant women

## **INTRODUCTION**

Promoting women's health is crucial for achieving the economic and social development goals of any society. According to a report by the World Health Organization, there are over 200 million pregnancies worldwide each year. Neglecting the healthcare needs of pregnant women can result in significant harm to both the individual and society as a whole. Therefore, it is essential to prioritize the health and well-being of this population.<sup>1</sup> During the initial trimester of pregnancy, many women experience nausea and vomiting, which is known as Nausea and Vomiting of Pregnancy (NVP). This is a prevalent and typical symptom that affects more than half of pregnant women, ranging from 50 to 90 percent. The first trimester is particularly suscepti-

ble to environmental factors that may impact the development of the fetus, making it a critical period.<sup>2</sup> Nausea is defined as an unpleasant and annoying condition in the gastric cavity preceding vomiting.<sup>3</sup>

The global prevalence of nausea and vomiting in pregnancy in 2017 was estimated to be about 35-91%<sup>4</sup> in world and its frequency in Iran is about 69.7%, of which 37.4% are mild nausea and vomiting, 46.7% moderate vomiting and 16% severe nausea and vomiting.<sup>5</sup> Nausea and vomiting of pregnancy usually begin at 4-6 weeks of gestation, peak at 8-12 weeks, and then decrease. In 9-20% of pregnant women, the symptoms continue after the 20th week of pregnancy and sometimes until the end.<sup>6</sup> For most people, these symptoms are mild and, while unpleasant, are not clinically significant. Nausea and vomiting of pregnancy if

left untreated can quickly turn into severe nausea and vomiting HG (Hyperemesis Gravidarum) and bring about many economic problems, malnutrition and gastrointestinal problems and in rare cases, serious nerve damage to the mother.<sup>7</sup> Nausea and vomiting in pregnancy have many complications for both the mother and the fetus.

In 0.5 to 2% of women, so severe can these symptoms be that they cause dehydration, electrolyte disturbance (hypokalemia), and weight loss of more than 0.05% (due to a lack of vitamins and minerals) and an urgent need for hospitalization and emergency measures. The dehydration and electrolyte disorders cause boredom, irritability, sadness, sleep disorders and confusion in women and have detrimental effects on their family, social and professional life.<sup>8</sup>

Understanding the main pathophysiology of nausea and vomiting is incomplete due to its unknown etiology, but generally it seems to be concomitant with the increase estrogen, progesterone with increasing human chorionic gonadotropin (larger placenta, female fetus, multiple pregnancies, and complete hydatidiform mole). Thyroid and adrenal hormones and serotonin, lack of vitamin B, digestive changes due to the enlargement of the uterus, individual, social, geographical and racial psychological factors are other causes of nausea and vomiting during pregnancy.9 Doctors and obstetricians use drug therapy to treat nausea and vomiting in many pregnant women.

Approximately 60% of gynecologists in Australia prescribe anti-nausea medications to manage this condition, but due to potential risks such as the thalidomide tragedy, many pregnant women are hesitant to use these medications.<sup>9</sup> There are many chemical drugs available to treat nausea and vomiting, and their benefits need to be carefully considered in light of potential risks associated with their use.<sup>10</sup> One of these drugs is ondansetron, which has maintained its position as the third line of treatment for nausea and the most frequently prescribed drug for the treatment of NVP and HG. The results of research on ondansetron have shown fetal defects such as heart and wall defects and in limited cases oral-facial defects such as cleft palate. Some herbal medicines, such as ginger, also contain active ingredients that increase the risk of bleeding by reducing platelet aggregation, which requires further studies on herbal ingredients.<sup>11</sup>

In general, proper NVP management seems to be essential in mild cases as well. In order to correctly diagnose the severity of the disease, each pregnant mother must be evaluated and supported individually. Support and counseling is the interaction between two or more people with the aim of promoting education and awareness and helping to solve problems with a positive effect on the individual. This support is often provided by professionals and can include training, counseling or problem solving.<sup>12</sup>

Among other methods of relieving the symptoms of pregnancy nausea and vomiting are acupuncture, acupressure, reflexology, osteopathy, etc. Acupuncture is a non-invasive, easy-to-use technique and a type of complementary medicine that is used to treat many problems and diseases by inserting a needle into specific anatomical points. The most used point for treating nausea and vomiting in acupressure is the P6 point. The location of this point is 2-3 fingers wide above the inner crease of the wrist and between the flexor tendons. By stimulating this point with a finger or a wide pointed object, nausea is relieved. Acupressure by the individual used without the need for a needle by applying constant pressure to the same specific points.<sup>13</sup> Pressure on these points has no side effects and according to studies in different geographical locations, the effect of acupressure on nausea and vomiting of pregnancy has not been the same and different results have been presented.14,15

Due to the high prevalence of nausea and vomiting of pregnancy and related complications, we decided to investigate the effect of acupressure and supportive counseling of the pregnant mother.

#### METHODOLOGY

The present study utilized a randomized double-blind controlled trial design with pre- and post-test measurements. The study population included all pregnant mothers between 8-12 weeks of gestation who were receiving care at healthcare centers in Hamadan, Iran, between September 2019 and June 2020. The study protocol was reviewed and approved by the Medical Research Ethics Committee of Hamadan University of Medical Sciences (IR.UMSHA.REC.1398.422). Written informed consent was obtained from all participants in the study. The study was conducted in accordance with applicable guidelines and regulations. The Sample size based on the information of Soltani et al. (2016), was obtained in that the standard deviation of vomiting frequency in both groups was  $\sigma_1 = 1.9$  and  $\sigma_2 = 2.9$ , respectively and  $(\mu_1 - \mu_2)$  $\mu_{21}$  =1.9 was at the first level error 0.05 and test power of 80% of required sample size in each group was 30.16

The study included pregnant women aged 18-35 with a single pregnancy, 8-12 weeks of gestational age, mild to moderate nausea and vomiting (score 4-12) based on PUQE-24 questionnaire, and no history of acupuncture or acupressure. Participants had to have no mental disorder or underlying disease, no smoking or alcohol use, and interest in attending counseling sessions. Exclusion criteria included interruption or non-completion of the study, abortion, bleeding, or severe nausea and vomiting. The study randomly selected one comprehensive health center from each of the four regions in Hamadan and included 15 participants from each center. The questionnaire employed in the study comprises of three questions that assess the frequency and severity of nausea and vomiting experienced by pregnant women over the last 24 hours, using a five-point Likert scale. Each question includes five options, which are scored from 1 to 5. The scoring system used in the study is as follows: scores ranging from 1 to 3 indicate the absence of nausea, while scores between 4 to 6 indicate mild nausea and vomiting. Scores in the range of 7 to 12 indicate moderate symptoms, and scores of 13 or above are classified as severe. Pregnant women were required to complete the questionnaire on a daily basis for three days before the start of the intervention and for a period of 14 days following the commencement of the study.

Researchers contacted eligible participants through the pregnancy care system, confirmed inclusion criteria, and assigned them randomly to two groups (Figure 1). Participants in group 1 received three face-toface support counseling sessions by a midwife one session every week, and continuous follow-ups by phone or online, using a 24hour P6 acupressure wristband for fourteen days, and the group 2 had the same number of sessions using a placebo wristband. The midwife who performed the intervention had received the necessary training in this field and obtained the relevant certificates. Having filled in the consent form to enter the study, they were entered into the desired group according to the letter in the envelope and after filling in the guestionnaire related to nausea and vomiting before the study, self-reportedly under the supervision of the researcher and ensure nausea score in the range of 4-12 according to the PUQE-24 questionnaire, with the coordination of the pregnant woman, specific dates for holding 3 face-toface counseling sessions were given to each for 45-60 minutes.

Counseling sessions were held in a quiet place and as far as possible away from environmental stimuli and the presence of other people. These sessions were organized based on free time of pregnant woman.

Before the sessions, another person, without explaining the types of wristbands, would give a wristband with a special code to the subjects and according to their group code, taught them how to use the wristbands for two weeks, 24 hours except for the times of bathing and ablution and the place of its use (three fingers above the crease of the wrist between the two ligaments of the carpi radialis and palmaris longus)17 with verbal explanations or the use of relevant images and photos, and gave a reminder mark with a pen on the spot. The handcuff with the push button and the placebo was exactly the same, and only the button was more prominent for the non-placebo acupressure group on one side.

Wristband was made of elastic cloth with protruding button on one side and button without significant protrusion on the other side for use in placebo acupressure group,. (Figure 2)

Counseling sessions were conducted according to GATHER principles (Greet, Ask, Tell, Help, Explain, and Return) and the content of counseling based on the nausea and vomiting protocol was provided in the Safe Mothers National Program Booklet. According to this booklet prepared by the Ministry of Health of Iran, the care of pregnant women during and after childbirth is done according to the guidelines and also the schedule. According to GATHER principles, the necessary training and counseling are given to the clients with the utmost respect and regularity. Counseling sessions based on the individual characteristics of the subjects continued to ensure their full understanding to implement the desired changes (Table1).

Finally, In order to blind the study, the person who collected the information and the person who performed the statistical analysis of the data did not know about the intervention and control group and the research subjects did not know the type of handcuff they received.

In this study, two questionnaires have been used: the first questionnaire consists of 3 parts, the first part including the demographic information, the second part including the midwifery information, the third part including the pregnancy nausea and vomiting, the validity of which was performed by Midwifery and nursing lecturers of Hamadan University of Medical Sciences with content validity method and the reliability of the guestionnaire was measured by re-testing of 20 pregnant women with inclusion criteria and the obtained information was entered into SPSS software version 21 and Cronbach's alpha coefficient of the whole questionnaire was r = 0.92 and based on each section: the first questionnaire: r = 0.93, the second questionnaire: r = 0.85 and the third questionnaire: r = 0.94, which was acceptable being more than 0.7.

The second part is the Pregnancy-Unique Quantification of Emesis and Nausea Questionnaire (PUQE-24), which is a modified PUQE tool for measuring the severity of nausea and vomiting (changing from 12 hours to 24 hours to take into account the time spent sleeping) and its validity and reliability have been confirmed in 2015 in YavariKia's study with Cronbach's alpha of 0.81%.<sup>18</sup> The data collected through the questionnaire was used to assess the effectiveness of the intervention on reducing the severity and frequency of nausea and vomiting in pregnant women.

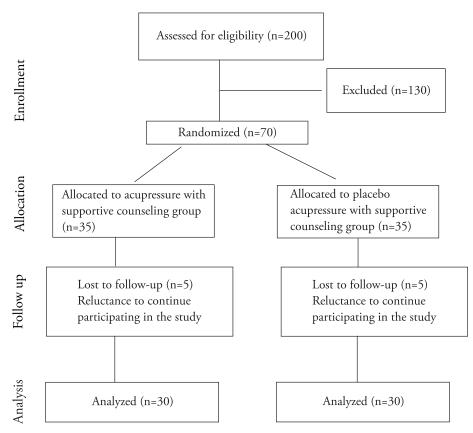
The data collected in this study was analyzed using SPSS statistical software (version 21). To determine the significance of the findings, various statistical tests including independent T-tests, Chi-square tests, and repeated measurement tests were employed, and a p-value of less than 0.05 was considered statistically significant.

### RESULTS

Two groups (group one: supportive counseling and acupressure wristbands and group two (1,2): supportive counseling and placebo wristbands) were not statistically different in terms of influential variables such as age ( $28.10\pm5.13$ ,  $27.93\pm4.71$  respectively), duration of marriage ( $5.93\pm4.19$ ,  $5.43\pm3.90$  respectively), body mass index ( $25.13\pm3.66$ ,  $24.77\pm4.66$  respectively), the time of onset of nausea ( $5.87\pm1.22$ ,  $5.83\pm1.41$  respectively) and gestational age( $9.63\pm1.42$ ,  $9.17\pm1.3$  respectively) at the time of the study were completely homogeneous (Table 2).

The results of pre-study comparisons showed that there was no statistically significant difference between the two groups regarding the education variable (P = 0.18). Most of the spouses of these women also had university education in both groups (P = 0.31). In both groups, most subjects were housewives (P = 0.48), their pregnancy was planned and desired (P = 0.59) and they had no history of infertility (P = 0.68).

The majority of the spouses of the subjects evaluated their information about pregnancy as "somewhat" and a small percentage of people in both groups had complete information about pregnancy and there was no statistically significant difference between the two groups in terms of this variable (P =0.21). Most of the subjects stated that they have a good relationship verbally, sexually and emotionally with their spouses and in this regard there was no significant difference between the two groups (P = 0.58, P = 0.33, P = 0.93, respectively). The rest of the participants evaluated good relationship to some extent and a very small percentage said that they do not have a good relationship with their spouses in any way. There is









no statistically significant difference between the two groups in terms of domestic violence (P = 0.058). The first group had a higher proportion (56.7%) of participants who had prior knowledge of effective methods to alleviate nausea, whereas in the second group, a larger percentage (40%) reported having no such information. However, the difference between the two groups in terms of knowledge regarding effective remedies was not statistically significant (p = 0.21). (Table3). erbate nausea, including food intake, social interactions, visiting specific locations, daily stress, or a combination of these factors, the majority of participants in both groups reported that certain types of food were a significant contributing factor to their nausea (41.4% in group one and 40.0% in group two). Notably, there was no statistically significant difference between the two groups in terms of the factors that were perceived to aggravate nausea (p = 0.68). (Table 4).

Among the various factors known to exac-

Analysis of variance of repeated mea-

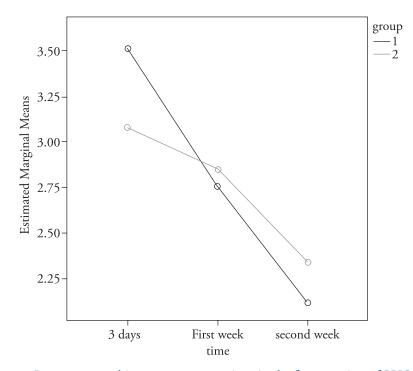
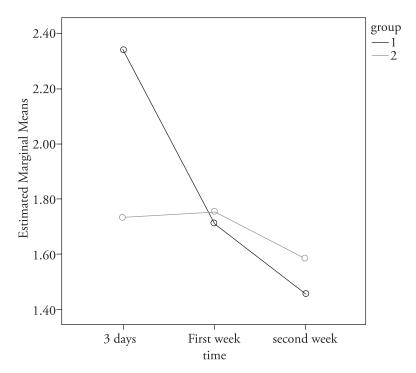


Figure 3: Intra-group and inter-group comparison in the first question of PUQE-24 questionnaire in three designated times



nificant separately, (P < 0.001), but there is no statistically significant difference between the two groups (P = 0.08). (Figure3)

For the second guestion of PUQE-24 questionnaire, which examines the mean score of number of vomiting times during the day for 17 consecutive days (Table 5), the analysis of variance of repeated measures showed that in general, each of the groups of supportive counseling and acupressure wristband and supportive counseling and placebo wristband separately, were effective in reducing the mean score of number of vomiting times and in general, this difference was statistically significant (P < 0.001). Comparison between the two groups showed that intervention in the first group made a greater decrease in the number of vomiting times than that in the second group, although this difference was not statistically significant (P = 0.78) (Figure 4).

According to the analysis of variance of repeated measures for the third question of PUQE-24 questionnaire (Table5), comparing the mean of group one (support counseling group and acupressure wristband) and group two (support counseling group and placebo wristband) in three days before the start of the study, the first week of the study and the second week of the study showed that the difference between the two groups was not significant (p=0.12) but the changes within each group were different and significant (p <0.001). During the daily study for fourteen days from the beginning of the study, the frequency of retching in both groups decreased while this reduction was more noticeable in supportive counseling and acupressure wristband (Figure5).

Figure 4: Intra-group and inter-group comparison in the second question of PUQE-24 questionnaire in three designated times

sures for the first question of the questionnaire (Table 5), which is the evaluation of the average duration of nausea or stomach problems in the day during three time periods (three days before the start of the study, the first week of the study and the second week of the study) in both groups showed changes of each group are statistically sig-

#### DISCUSSION

The objective of this study was to evaluate the effectiveness of face-to-face supportive counseling provided by midwives, as well as the use of acupressure and placebo

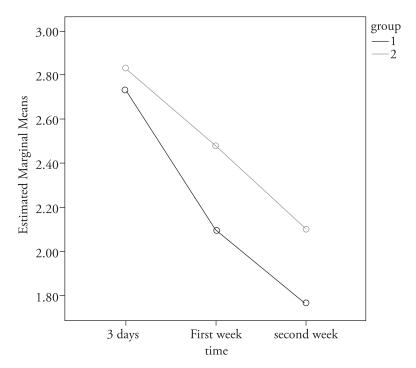


Figure 5: Intra-group and inter-group comparison in the third question of PUQE-24 questionnaire in three designated times

Table	1: Content	of training	sessions b	ased on	CATHER	principles
Table	1: Content	or training	sessions D	ased on	GAINER	principles

wristbands, in reducing the severity of nausea and vomiting in pregnant women. The findings of this study revealed that participation in supportive counseling sessions, as well as the use of acupressure wristbands (group 1), had a significantly greater effect in reducing the duration of nausea, vomiting, and retching compared to the use of placebo wristbands (group 2). According to this purpose, our study has been in line with Isbir (2016)<sup>4</sup>, Soltani (2017)<sup>16</sup>, Abedian (2014)<sup>19</sup>, Maltepe (2015)<sup>20</sup> 's studies. The results of these studies have shown the effect of counseling during pregnancy in reducing nausea and vomiting during this period. Counseling regarding nutritional, medicinal and lifestyle changes in patients suffering from nausea and severe vomiting during pregnancy leads to more favorable results in the pregnancy outcomes of these mothers. In the conducted studies, the content of counseling is based on changes in diet and medication and lifestyle, which is closer to the content of counseling in the present study.

Session	Content	GATHER	Consulting method	Duration
Session 1	Introducing and welcoming, establishing a sincere relationship with a preg- nant woman 2. Asking about living conditions and examining the factors that provoke nausea in a pregnant woman 3. Encouragement to participate in the next counseling session. 4. Answering subjects' questions	Greet, Ask	Face –to –face consulting with pregnant women	45-60 min
Session 2	<ol> <li>Reviewing the contents of the previous session 2. Giving information about the factors affecting nausea and vomiting and its soothing methods</li> <li>Helping pregnant women to use appropriate methods to relieve nausea and vomiting symptoms based on the protocol and booklet of the National Program for Safe Mothers 4. Encouraging them to participate in the next consultation session</li> </ol>	Tell, Help	Face –to –face consulting with pregnant women	45-60 min
Session 3	<ol> <li>Reviewing the contents of the previous session 2. Full explanation of the selected methods to reduce nausea 3. Following up the pregnant mother for the next appointments. 4. Answering subjects' questions</li> </ol>	Explain, Return	Facetoface consulting with pregnant women	45-60 min

#### Table 2: Comparison of quantitative demographic variables between two groups

Variable	Group 1	Group 2	p-value*
Age,Year	28.10 (5.13)	27.93 (4.71)	0.89
Spouse's age,Year	33.3 (6.06)	32.60 (5.0)	0.62
Age difference, Year	5.53 (3.82)	4.83 (3.47)	0.46
BMI, Kg/m2	25.13 (3.66)	24.77 (4.66)	0.72
Number of marriages	1.10 (0.3)	1.07 (0.2)	0.88
Duration of marriage, Year	5.93 (4.19)	5.43 (3.90)	0.63
Start of vomiting,Week	5.87 (1.22)	5.83 (1.41)	0.91
Gestational age,Week	9.63 (1.42)	9.17 (1.34)	0.19
Number of pregnancies	0.87 (0.9)	0.83 (0.95)	0.97

Variable	Group 1	Group 2	p-value*	X <sup>2</sup>	
	yes	6 (20.0)	6 (20.0)		
Level of spouse information about pregnancy	no	13 (43.3)	7 (23.3)	0.21	3.08
	relatively	11 (36.7)	17 (56.7)		
	yes	21 (70.0)	21 (70.0)		
Appropriate verbal communication	no	1 (3.3)	0 (0.0)	0.58	1.05
	relatively	8 (26.7)	9 (30.0)		
	yes	17 (56.7)	22 (73.3)		
Sexual relationship	no	3 (10.0)	1 (3.3)	0.33	2.1
	relatively	10 (33.3)	7 (23.3)		
	yes	22 (73.3)	20 (66.7)		
Emotional relationship	no	1 (3.3)	1 (3.3)	0.93	0.1
	relatively	7 (27.6)	9 (30.0)		
	yes	1 (3.3)	7 (23.3)		
Domestic violence	no	23 (76.7)	20 (66.7)	0.058	5.70
	relatively	6 (20.0)	3 (10.0)		
	yes	3 (10.0)	7 (23.3)		3.06
Level of information about nausea reduction methods	no	10 (33.3)	12 (40.0)	0.21	
	relatively	17 (56.7)	11 (36.7)		

Table 3: Comparison of (level of spouse information about pregnancy, appropriate verbal, sexual and emotional communication with spouse, level of domestic violence and level of information about nausea reduction methods) in both groups

Table 4: Comparison of nausea exacerbating variables in both groups

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Factors of nausea aggravation	Group 1 (%)	Group 2 (%)	Statistical test result
Food consumption	12 (41.4)	12 (40.0)	
Relation with relatives and friends	2 (6.7)	2 (6.7)	
Entry to specific places	1 (3.4)	0 (0/0)	
Daily stresses	0 (0/0)	2 (6.7)	x <sup>2</sup> =6.55
Food stuff and relation with specific persons	6 (20.7)	5 (16.7)	*p= 0.68
Food stuff and entry to special places	4 (13.8)	3 (10.0)	
Food stuff and stress	2 (6.9)	4 (13.3)	
A set of factors	3 (10.3)	2 (6.6)	

In Isbir's study, the consultations were not based on personal needs and were based on dietary and lifestyle changes. But in the present study, counseling sessions were conducted based on individual and family characteristics and the living conditions of mothers. Identifying the triggers of mother's nausea and vomiting by the consultant has helped a lot in achieving the goals of this study. Due to the influence of psychological factors in the occurrence of nausea and vomiting during pregnancy, psychological support can reduce the symptoms of this problem in most cases. And in general, counseling, whether by phone or in person, plays an important role in reducing problems during pregnancy, especially nausea and vomiting.

Another goal of this study was the effect of acupressure on P6 point on reducing nausea and vomiting during pregnancy. The results of the present study were in line with the results of Adlan<sup>13</sup> (2017), Negarandeh<sup>21</sup> (2020) 's studies. Alternative medicine offers several methods that can be used to alleviate the symptoms of nausea and vomiting during pregnancy. Acupressure, a form of therapy related to acupuncture, is an effective method that has been used extensively in traditional Chinese medicine for the treatment of nausea and vomiting. Although

the exact mechanism by which acupressure therapy reduces nausea and vomiting is not yet fully understood, it is a widely accepted practice for the management of these symptoms in pregnant women. But the possible mechanism of its effect is the release of beta-endorphins in the cerebrospinal fluid and its effect on µ receptors and decreased stomach movements. Stimulating the P6 point may cause the release of this neurochemical substance. It is believed that sensory impulses resulting from nerve stimulation are transmitted to the brain stem in a vague way and prevent nausea and vomiting. And as long as pressure is applied to the P6 point, the center of nausea and vomiting in

Questionaries'	item PUQE-24	Three days before	The first week	The second week	Total days	p-value*	F
	Group1	3.51 (0.92)	2.75 (0.94)	2.11 (1.01)	2.43 (0.93)	>0.001	
Q1	Group2	3.07 (0.92)	2.85 (0.72)	2.33 (0.57)	2.59 (0.58)	>0.001	0.48
	**p-value	0.07	0.66	0.29	0.43		
	Group1	2.33 (0.87)	1.71 (0.68)	1.45 (0.38)	1.58 (0.50)	0.001>	
Q2	Group2	1.73 (0.58)	1.75 (0.62)	1.58 (0.48)	1.66 (0.50)	0.001>	0.30
	p-value**	0.003	0.82	0.26	0.52		
	Group1	2.73 (0.78)	2.09 (0.85)	1.76 (0.75)	1.92 (0.77)	0.001>	
Q3	Group2	2.83 (0.88)	2.48 (0.76)	2.10 (0.75)	2.29 (0.63)	>0.001	0.12
	**p-value	0.65	0.07	0.07	0.05		

Table 5: Comparison of Mean (SD) of the indexes of PUQE-24 questionnaire between and within groups with repeated measur	-
ment test	

the brain becomes insensitive, and when the pressure is removed, the therapeutic effect is removed after 24 hours. The combined use of acupressure and supportive counseling has been investigated in Saeedi 's study (2019)<sup>14</sup> on reducing nausea and vomiting during pregnancy.Non-generalizability due to less sample size and non-randomization are the limitations of the study, therefore it considered as bias.

## CONCLUSION

The result of the present study shows that the use of acupressure on the point P6, along with midwifery consultation, is one of the most effective and uncomplicated methods in the treatment of nausea and vomiting during pregnancy. And counseling with pregnant women has a great effect on the treatment of pregnancy nausea and vomiting. Thus the use of this method is recommended in the treatment of affected women.

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#### Author's Contribution

AAM, SZM, BK, and PP were involved in designing the study, collecting the data, and writing the manuscript. GR contributed to the study design and data analysis. Authors agree to be accountable for all aspects of the work in ensuring that questions related to the accuracy or integrity of any part of the work are appropriately investigated and resolved.

#### **Conflict of Interest**

Authors declared no conflict of interest

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None

# Data Sharing Statement

The data that support the findings of this study are available from the corresponding author upon reasonable request.