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Effects of prenatal yoga on women's stress and immune function across pregnancy: A randomized controlled trial

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ABSTRACT

Objective: The effects of prenatal yoga on biological indicators have not been widely studied. Thus, we compared changes in stress and immunity salivary biomarkers from 16 to 36 weeks' gestation between women receiving prenatal yoga and those receiving routine prenatal care.
Design: For this longitudinal, prospective, randomized controlled trial, we recruited 94 healthy pregnant women at 16 weeks' gestation through convenience sampling from a prenatal clinic in Taipei. Participants were randomly assigned to intervention (n = 48) or control (n = 46) groups using Clinstat block randomization.
Intervention: The 20-week intervention comprised two weekly 70-min yoga sessions led by a midwife certified as a yoga instructor; the control group received only routine prenatal care.
Main outcome measures: In both groups, participants' salivary cortisol and immunoglobulin A levels were collected before and after yoga every 4 weeks from 16 to 36 weeks' gestation.
Results: The intervention group had lower salivary cortisol ($p < 0.001$) and higher immunoglobulin A ($p < 0.001$) levels immediately after yoga than the control group. Specifically, the intervention group had significantly higher long-term salivary immunoglobulin A levels than the control group ($p = 0.018$), and infants born to women in the intervention group weighed more than those born to the control group ($p < 0.001$).
Conclusion: Prenatal yoga significantly reduced pregnant women's stress and enhanced their immune function. Clinicians should learn the mechanisms of yoga and its effects on pregnant women. Our findings can guide clinicians to help pregnant women alleviate their stress and enhance their immune function.

1. Introduction

During pregnancy, women undergo bio-physio-psycho-social changes that may cause stress.[1,2] This pregnancy-related stress can increase when pregnant women also experience stressful events such as the death of a loved one, family illness, divorce, work load or loss of employment.[3] These external stressors can lead to adverse perinatal outcomes[3,4] such as perinatal depression,[1,5,6] postpartum depression,[7] as well as pregnancy-induced hypertension and preeclampsia.[8] Prolonged maternal stress during pregnancy has also been related to adverse birth outcomes such as premature and low birth-weight infants,[9] contraction of uterine artery blood,[10] and abnormal fetal brain development.[2,11,12] Excessive maternal stress during pregnancy has also been associated with children's later attention deficit hyperactivity disorder[13] or lower executive-function performance.[12]

The stress response is modulated by the hypothalamic–pituitary–adrenal (HPA) axis, in which the hypothalamus produces corticotropin-releasing factor that stimulates the pituitary to produce adrenocorticotropin, in turn leading to adrenal secretion of cortisol.[14,15] During pregnancy, the placenta

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also produces corticotropin-releasing factor, increasing adrenal secretion of cortisol even more.[11] Cortisol levels rise continuously after 15 weeks' gestation and fall abruptly after delivery.[16]

To prevent adverse outcomes, pregnant women's stress should be managed and interventions should be provided to reduce elevated cortisol levels. Promoting maternal health is important because it determines fetal and infant health, consistent with US[17,18] and global[19] goals to promote maternal, fetal, and infant health. Good maternal health can help reduce public health challenges for families, communities, and health care systems by preventing preterm birth or child disability.[18] Furthermore, good mental health predisposes mothers to have better interactions with their children to stimulate their development, thus helping them reach their full developmental potential.[20] In contrast, children of mothers with poor mental health have significantly delayed development.[20] Therefore, promoting maternal, fetal, and infant health requires appropriate interventions to alleviate women's stress before, during, and after pregnancy.

However, pharmacological stress management may not be acceptable for pregnant women due to concern about the potential teratogenic effects of some commonly used pharmaceuticals, such as barbiturates, opioids, benzodiazepines, thalidomide, and paroxetine. Indeed, use of these drugs by pregnant women has been associated with increased risk for severe fetal limb defects and organ dysgenesis.[21,22] Therefore, physicians should encourage pregnant women to avoid taking over-the-counter drugs, explain the teratogenic effects of these drugs, and provide guidelines for safely using these drugs.[22]

Thus, non-pharmacological stress-relief interventions are important during pregnancy to promote maternal and fetal health and improve perinatal outcomes. Non-pharmacological interventions shown to relieve prenatal stress include music therapy,[23] cognitive-behavioral intervention,[24] aromatherapy,[25] yoga,[6,26–31] and relaxation techniques.[32] However, most of these studies, including nine of 10 randomized controlled trials in a systematic review [29] of yoga's effects on pregnant women, measured intervention effects on pregnant women's stress by self-report questionnaires. Another four studies measured yoga effects on heart rate fluctuation.[25,26,28,32] Nine studies in a systematic review found that practicing yoga during pregnancy relieved stress; reduced anger, anxiety and depression; and improved birth outcomes.[29] Among all studies we reviewed, only four measured effects on salivary cortisol levels after yoga.[6,27,30,31] Cortisol is considered a biomarker of both psychological and physical health.[33] Furthermore, stress-associated increases in cortisol levels may reduce cellular immunity, increasing risk of infection[4] and inhibiting secretion of salivary immunoglobulin A (IgA).[34–36]

Yoga, a type of mind-body-spirit relaxation exercise has been established as a potentially powerful intervention.[37] Indeed, yoga postures can be modified to fit pregnant women's competence. Practicing yoga has been proposed to modulate the HPA axis by buffering cortisol release in response to stress,[38] and is considered to keep women relaxed during pregnancy.[28,29,39,40] Yoga also improves perinatal outcomes,[39] i.e., fewer prenatal disorders and premature births as well as less labor pain,[29] stress,[26,29,41] anxiety,[30,42] depression,[6,27,30,42] and pregnancy-related lumbopelvic pain.[43] However, pregnant women who practiced yoga at home were reported to have an increased incidence of postnatal/intrapartum hemorrhage possibly associated with non-anemic low iron,[44] suggesting that yoga can negatively affect birth outcomes. These inconsistent findings emphasize the need for further studies on prenatal yoga effects on pregnant women and birth outcomes.

Four studies reviewed above found that salivary cortisol levels were lower after pregnant women regularly practiced yoga in the US,[6,27] the UK,[30] and Japan.[31] However, none of these studies measured the effect of yoga on pregnant women's IgA levels. Furthermore, no studies explored the effects of practicing yoga on pregnant Taiwanese women's biomarker levels. Thus, more information is needed on yoga's effects on pregnant Taiwanese women's IgA levels.

To fill these gaps in knowledge, we designed this study to evaluate the effects of prenatal yoga on women's stress and immune biomarkers across pregnancy. Specifically, we measured monthly levels of salivary cortisol and IgA from 16 to 36 weeks of pregnancy. The framework to support the yoga intervention was the cognitive-behavioral model of relaxation.[45] This model emphasizes three components of relaxation: (1) reducing arousal or inducing a relaxation response, (2) developing cognitive-relaxation skills by focusing attention, passively letting go of stress, and becoming receptive to peacefulness, and (3) increasingly acquiring cognitive structures that support relaxation.[45] Yoga helps pregnant women release stress by focusing on stretching, deep breathing (focusing the mind), and guided imagery.[45,46]

Based on the mechanisms by which yoga affects stress[45] and the literature,[38,45] we hypothesized that pregnant women practicing yoga would have lower salivary cortisol and higher salivary IgA levels than pregnant women receiving routine prenatal care.

2. Methods

2.1. Design and randomization

For this prospective randomized control trial with a longitudinal, repeated-measures design, pregnant participants were randomly assigned by a blinded statistician using Clinstat block randomization[47] to the control and intervention groups. From 16 to 36 weeks' GA, participants in the control group received only routine prenatal care, and those in the intervention group received routine prenatal care plus the yoga intervention (see Section 2.4). This study followed the CONSORT guidelines.[48]

2.2. Participants and setting

Pregnant women around 16 weeks' GA were recruited by convenience sampling from February 2014 through February 2015 from the prenatal clinic of a medical center in Taipei. Women were included if they met these criteria: (1) normal pregnancy, (2) 20–45 years old, (3) agreed to follow-up collections of saliva samples, 4) could read and write Chinese, (5) if in the intervention group, willing to attend at least 85% of yoga sessions (34 sessions), and (6) able to abstain from eating, drinking caffeine-containing beverages, and engaging in strenuous physical activity for 2 h before saliva collection. Women were excluded by these criteria: (1) taking oral steroids, (2) history of severe illness (i.e., heart disease, systemic lupus erythematosus, metabolic disorders), or depression, (3) using drugs (prescribed or illicit), and (4) high-risk pregnancy (i.e., first-trimester vaginal bleeding, incompetent cervix or cervical cerclage, artificially inseminated, multiple gestations, fetal growth restriction or other abnormalities). Of 136 pregnant women screened, 115 met the study criteria (Fig. 1). Participation was refused by 14 women who could not follow our study plan; thus, the final sample included 101 pregnant women. With each participant's permission, we checked her prenatal screening status with her obstetrician to confirm her study eligibility. In the intervention group, two women dropped out due to moving and placenta previa. In the control group, five women dropped out due to moving ($n = 1$), preeclampsia ($n = 2$), and gestational diabetes ($n = 2$). Pregnant women who did and did not complete the study did not differ significantly in any demographic characteristics measured. Study power was estimated using G*Power version 3.1.9.2.[49] Our a priori power analysis

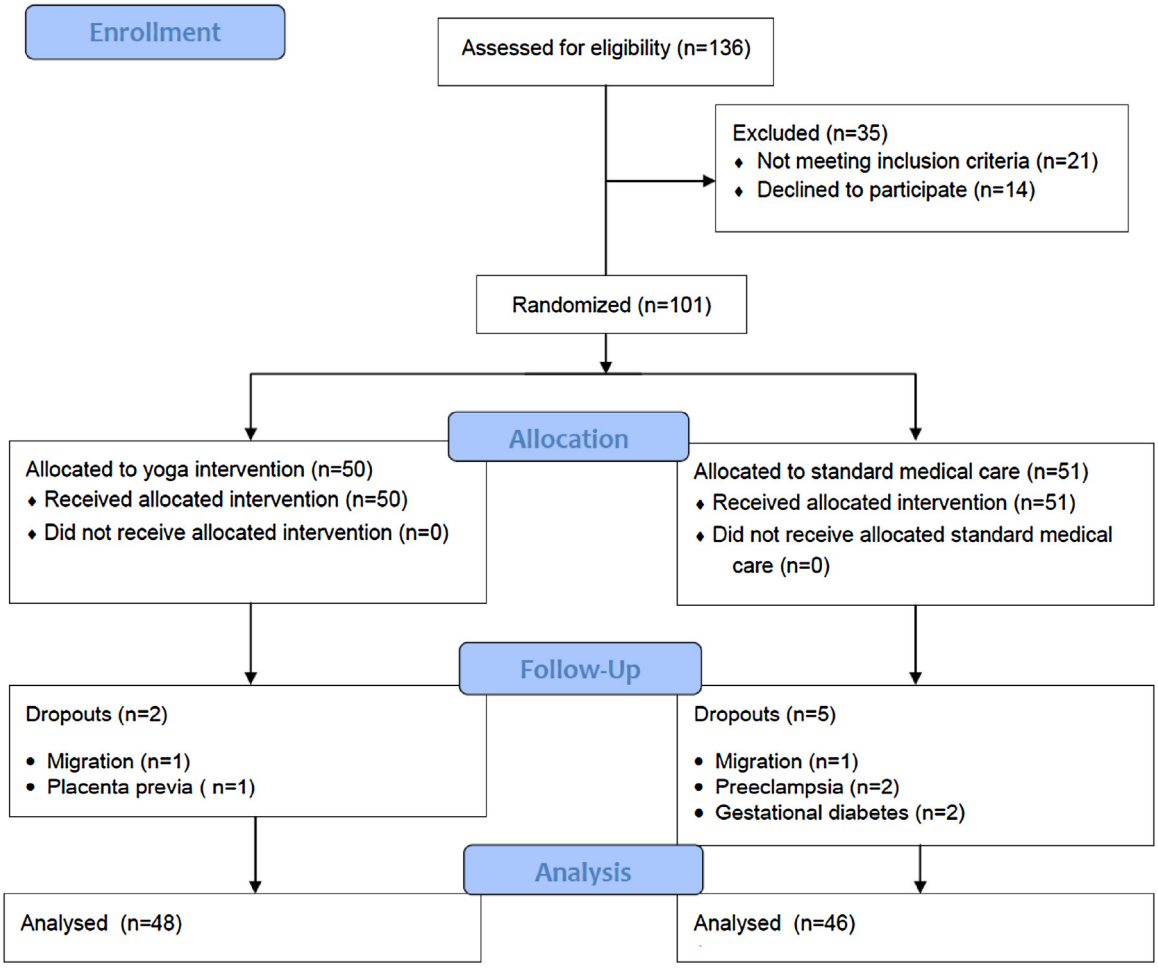

Fig. 1. Study design and participant flow chart.

for repeated measures within factors showed that the powers for cortisol and IgA were 0.99 and 0.86, respectively.

2.3. Procedures

Before data were collected, this study was approved by the institutional review board (IRB) of Tri-Service General Hospital (IRB No.2-102-05-148). Pregnant women who met the study criteria received a pamphlet introducing the study when they first came to the prenatal clinic. Interested women returned the response sheet to a research assistant whose name was on the sheet; the research assistant then explained the study in more detail and obtained the women's consent. Based on block randomization, each participant had the same probability to be assigned to the control or intervention group. Routine prenatal care was provided in a quiet room next to the prenatal clinic by a research nurse with about 2 years' obstetric work experience. This nurse was trained by the principal investigator (JJL) to provide routine prenatal care to all participants.

For participants in the intervention group, saliva samples were collected by the first author 10 min before and immediately after yoga. To account for circadian variations in salivary cortisol and IgA,[50,51] yoga was scheduled between 14:30 and 15:40. Saliva samples were collected from control-group participants by the research nurse at the same times as for intervention-group participants. Data from the first saliva samples were used as baseline data.

2.4. Prenatal yoga intervention

Prenatal yoga was offered in six 70-min yoga sessions per week for 20 weeks, with 10–12 women in each session. Participants attended two sessions of yoga each week, held in a quiet room near the prenatal clinic. Yoga was specifically designed for women during the second and third trimesters and was guided by a former midwife certified as a prenatal yoga instructor for more than 20 years. Yoga included physical postures/stretching, deep breathing, guided imagery, and deep relaxation. Before each posture/stretch, the instructor guided participants to relax each body part and calm their mind.[27,28,39] The instructor demonstrated each posture/stretch based on pregnant women's needs and tolerance with aids (e.g., blankets, cushions, blocks, and chairs) to support balance. Guided imagery helped women focus on their breathing to gain inner peace. Breathing involved deeply inhaling and exhaling slowly through alternating nostrils. At the end, the instructor guided participants to lie on their left side for deep relaxation to facilitate sleep and physio-psycho-spiritual calmness. Participants could quit yoga at any time if they showed any signs of discomfort, abnormal bleeding, or uterine contractions.

2.5. Saliva collection

Two saliva samples (before and after yoga) were collected from participants in both groups on each data collection day at 16, 20, 24, 28, 32 and 36 weeks' GA (12 saliva samples/participant).

Table 1
Participants' characteristics by group (n = 94).

Variable	Intervention group (n = 48) n (%)	Control group (n = 46) n (%)	Total (n = 94) n (%)	p-Value
Gravida[a]				0.681
1	29(60.42)	27(58.70)	56(59.57)	
2	13(27.08)	16(34.78)	29(30.85)	
>3	6(12.50)	3(6.52)	9(9.58)	
Para[a]				0.239
Primipara	36(75.00)	36(78.26)	72(76.60)	
Multipara	12(25.00)	10(21.74)	22(23.40)	
Education level[a]				0.968
College	5(10.42)	4(8.70)	9(9.57)	
University	29(60.42)	29(63.04)	58(61.70)	
Graduate school	14(29.16)	13(28.26)	27(28.73)	
Employment status[a]				0.738
Full-time homemaker	19(39.58)	15(32.61)	34(36.17)	
Employed full-time	29(60.42)	31(67.39)	60(63.83)	
Annual household income[a] (NTD)				0.71
<600,000	8(16.67)	8(17.39)	16(17.02)	
600,000–800,000	8(16.67)	6(13.04)	14(14.89)	
800,000–1,000,000	7(14.58)	9(19.57)	16(17.02)	
>1,000,000	25(52.08)	23(50.00)	48(51.07)	
Exercise habits[a]				0.079
Yes	18(37.50)	13(28.26)	31(32.98)	
No	30(62.50)	33(71.74)	63(67.02)	
Family psychiatric history[a]				0.777
Yes	4(8.33)	5(10.87)	9 (9.57)	
No	44(91.67)	41(89.13)	85(90.43)	
	M ± SD	M ± SD	M ± SD	
Chronological age[b] (years)	33.1 ± 4.02	32.9 ± 3.66	33.0 ± 3.83	0.808
Gestational age[b] (years)	39.3 ± 0.71	38.6 ± 1.32	38.9 ± 1.04	0.014
Birth weight[c] (grams)	3170.5 ± 240.54	3048.2 ± 269.67	3124.3 ± 258.81	<0.001

NTD: New Taiwan dollars (33 NTD equals US $1).
[a] Fisher's exact test.
[b] Mann–Whitney *U* test.
[c] Independent group *t*-tests.

Participants were advised verbally and in writing to (1) avoid alcohol for 24 h, (2) not eat, brush teeth, or consume caffeine products within 2 h before saliva collection,[52] and (3) to avoid collecting saliva if their gums were bleeding. To collect a sufficient quantity of saliva, participants chew a cotton swab (Salimetrics, State College, PA, USA) for about 2–3 min, thus stimulating saliva flow to a sufficient amount (1–2 ml). After the collection tube was centrifuged at 1000 × *g* for 2 min, saliva was stored at −80 °C until assay.

2.6. Saliva assay

The concentrations of cortisol (μg/dL) and IgA (μg/mL) in saliva samples were analyzed by the first author, who had received 2 years of training in medical scientific laboratories. Cortisol was measured using a competitive enzyme-linked immunoassay (ELISA) kit (Cayman Chemical Company, USA), and IgA was measured using double-antibody sandwich ELISA method per the manufacturer's instruction (ICL, Inc., USA). The concentrations of cortisol and IgA in test samples were extrapolated from standard curves constructed from known concentrations of cortisol and immunoglobulin standards, respectively and corrected for sample dilution. Salivary samples were washed before assay per kit instructions, and sample absorbance was measured at 450 nm in ELISA Reader (Biotek, USA).

2.7. Statistical analysis

Data were analyzed using SPSS Version 20.0. All participants' demographic and clinical characteristics were compared by independent *t*-test (or Mann–Whitney *U* test, as appropriate) for continuous variables and Fisher's exact test for categorical variables. Data were described by means and standard deviation (SD) for continuous variables, and frequencies and percentage for categorical variables. To evaluate immediate (posttest) and long-term (weeks) effects of yoga, we used the generalized estimating equation (GEE) method's generalized linear models with 3-way interaction (group × weeks × post) to compare salivary cortisol and IgA levels between the intervention and control groups (group).[53] Statistical significance was defined as $p < 0.05$.

3. Results

3.1. Participants' characteristics

Our sample of 94 pregnant women (48 in the intervention group, 46 in the control group) did not differ significantly in gravida, para, education level, employment status, annual household income, exercise habits, family history of psychiatric disease, and mean chronological age, which was 33.0 ± 3.8 years (range = 24–43) (Table 1). However, the two groups differed significantly in GA ($p = 0.014$) and their infants' birth weight ($p < 0.001$). Women in the intervention group delivered infants at a mean GA of 39.3 ± 0.7 weeks and with mean birth weight of 3170.5 ± 240.54 g. Women in the control group delivered infants at a mean GA of 38.6 ± 1.3 weeks and mean infant birth weight of 3048.2 ± 269.67 g (Table 1).

3.2. Effects of prenatal yoga on salivary cortisol levels

At pretest (GA = 16 weeks), salivary cortisol levels for the intervention and control groups were not significantly different (Table 2). In the control group, the pretest-posttest difference (immediate effect) in salivary cortisol levels at 16 weeks' GA was not significant (Table 2; Fig. 2). However, in the intervention group, the pretest-posttest difference in salivary cortisol at 16 weeks' GA was significantly different (group × post interaction term, Table 2),

Table 2
Comparison of prenatal yoga effects on salivary cortisol by GEE (n = 94).

Variable	B	SE	Wald Chi-square	p-Value	95% Confidence Interval	
					Lower	Upper
Cortisol (μg/dL)						
Group[a] (1 vs. 0)	−0.03	0.04	0.53	0.469	−0.10	0.05
Post[b] (1 vs. 0)	0.02	0.01	3.08	0.079	−0.01	0.05
36 weeks vs. 16 weeks[c]	0.13	0.04	12.71	<0.001	0.06	0.20
32 weeks vs. 16 weeks[c]	0.08	0.04	3.24	0.072	−0.01	0.17
28 weeks vs. 16 weeks[c]	0.05	0.04	1.98	0.159	−0.02	0.13
24 weeks vs. 16 weeks[c]	0.01	0.03	0.07	0.795	−0.05	0.07
20 weeks vs. 16 weeks[c]	−0.02	0.03	0.04	0.578	−0.08	0.04
Group × 36 weeks[c]	0.04	0.05	0.78	0.379	−0.05	0.13
Group × 32 weeks [c]	0.06	0.06	1.21	0.271	−0.05	0.17
Group × 28 weeks[c]	0.01	0.04	0.05	0.823	−0.08	0.10
Group × 24 weeks[c]	0.01	0.04	0.02	0.886	0.07	0.08
Group × 20 weeks[c]	0.04	0.04	1.01	0.314	−0.04	0.12
Group[a] × post[b]	−0.13	0.02	55.84	<0.001	−0.16	−0.09
36 weeks[c] × post[b]	−0.03	0.01	7.25	0.007	−0.05	−0.01
32 weeks[c] × post[b]	−0.03	0.01	6.05	0.014	−0.05	−0.01
28 weeks[c] × post[b]	−0.00	0.01	0.01	0.934	−0.02	0.02
24 weeks[c] × post[b]	−0.03	0.01	8.07	0.005	−0.05	−0.01
20 weeks[c] × post[b]	−0.02	0.01	1.81	0.179	−0.04	0.01
Group × 36 weeks[c] × post[b]	−0.03	0.02	2.51	0.113	−0.08	0.01
Group × 32 weeks[c] × post[b]	−0.03	0.03	1.30	0.254	−0.078	0.02
Group × 28 weeks[c] × post[b]	0.01	0.02	0.15	0.697	−0.03	0.04
Group × 24 weeks[c] × post[b]	0.04	0.02	4.86	0.027	0.01	0.07
Group × 20 weeks[c] × post[b]	0.02	0.02	0.81	0.370	−0.02	0.06

GEE: Generalized estimating equations.
[a] Group: 1 = intervention group; 0 = control group.
[b] Post: 1 = posttest; 0 = pretest.
[c] The reference category was baseline or 16 weeks of gestation.

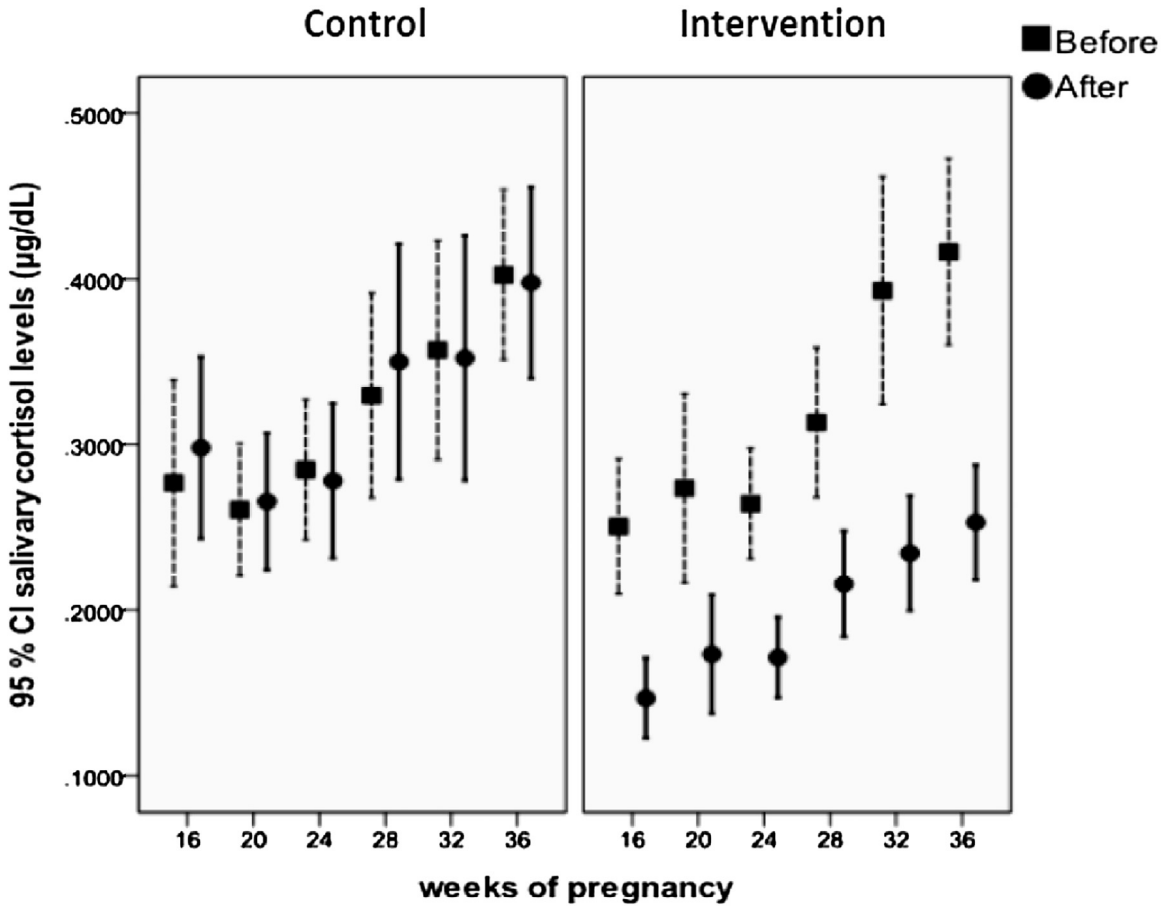

Fig. 2. Overall differences between the intervention and control groups in salivary cortisol (μg/dL) levels. Changes in salivary cortisol levels at six times during pregnancy for women receiving yoga (intervention group) or routine prenatal care (control group).

Table 3
Comparison of prenatal yoga effects on salivary Immunoglobulin A by GEE (n = 94).

Variable	B	SE	Wald Chi-square	p-Value	95% Confidence Interval	
					Lower	Upper
Immunoglobulin A (μg/mL)						
Group[a] (1 vs. 0)	1.73	8.69	0.04	0.842	−15.30	18.76
Post[b] (1 vs. 0)	1.17	1.82	0.41	0.521	−2.40	4.73
36 weeks vs. 16 weeks[c]	1.07	6.89	0.02	0.877	−12.44	14.58
32 weeks vs. 16 weeks[c]	11.88	5.49	4.66	0.031	1.10	22.64
28 weeks vs. 16 weeks[c]	5.00	4.66	1.15	0.283	−4.12	14.13
24 weeks vs. 16 weeks[c]	18.33	5.75	10.18	<0.001	7.07	29.60
20 weeks vs. 16 weeks[c]	− 2.77	4.48	0.38	0.537	−11.54	6.01
Group × 36 weeks[c]	27.27	11.52	5.61	0.018	4.70	49.84
Group × 32 weeks[c]	−6.33	8.81	0.52	0.473	−23.60	10.95
Group × 28 weeks[c]	5.67	8.92	0.40	0.525	−11.83	23.16
Group × 24 weeks[c]	−9.71	9.55	1.03	0.309	−28.43	9.01
Group × 20 weeks[c]	12.04	7.83	2.37	0.124	−3.30	27.38
Group[a] × post[b]	57.35	6.38	80.87	<0.001	44.85	69.85
36 weeks[c] × post[b]	−0.37	2.42	0.08	0.783	−5.45	4.11
32 weeks[c] × post[b]	−5.50	2.19	6.71	0.010	−9.98	−1.38
28 weeks[c] × post[b]	−3.40	2.81	1.62	0.203	−9.10	1.93
24 weeks [c] × post [b]	−1.50	2.41	0.39	0.533	−6.22	3.22
20 weeks[c] × post[b]	−0.47	2.02	0.07	0.793	−4.47	3.41
Group × 36 weeks[c] × post[b]	18.97	16.25	1.36	0.246	−12.89	50.83
Group × 32 weeks[c] × post[b]	−4.37	8.35	0.28	0.598	−20.75	11.96
Group × 28 weeks[c] × post[b]	−2.18	9.43	0.05	0.817	−20.67	16.30
Group × 24 weeks[c] × post[b]	−9.58	9.14	1.10	0.294	−27.49	8.32
Group × 20 weeks[c] × post[b]	−2.87	6.97	0.17	0.681	−16.52	10.78

GEE: Generalized estimating equations.
[a] Group: 1 = intervention group; 0 = control group.
[b] Post: 1 = posttest; 0 = pretest.
[c] The reference category was baseline or 16 weeks of gestation.

i.e., 0.13 unit lower than that of the control group ($p < 0.001$) across pregnancy. The immediate effect in the intervention group at 16 weeks' GA persisted at 20, 24, 28, 32, and 36 weeks' GA (Fig. 2; 3-way interaction terms, Table 2). Comparing the time effects of yoga on salivary cortisol levels at different times, we found that the control-group pretest salivary cortisol level at 36 weeks' GA was significantly higher than that at 16 weeks' GA (Table 2, 36 weeks *vs.* 16 weeks, $p < 0.001$) and approached borderline significantly higher at 32 weeks' GA ($p = 0.072$). In contrast, we found no significant time effects in the intervention group in salivary cortisol levels before yoga at each data collection time *vs.* baseline ($p = 0.271–0.886$) (Table 2; Fig. 2).

3.3. *Effects of prenatal yoga on salivary IgA levels*

At baseline (GA = 16 weeks), pretest values of salivary IgA levels did not differ significantly between the intervention and control groups (Table 3). In the control group, the baseline pretest-posttest difference (immediate effect) in salivary IgA levels was not significant (Table 3; Fig. 3). In contrast, the baseline pretest-posttest difference of the intervention group was significant ($p < 0.001$; group × post interaction term, Table 3). Specifically, the baseline pretest-posttest difference was 57.35 units significantly higher in the intervention group than in the control group. This immediate effect in the intervention group was maintained at 20, 24, 28, 32, and 36 weeks' GA (Fig. 3; 3-way interaction terms, Table 3, $p = 0.246–0.817$).

Comparing the time (or long-term) effects of yoga on salivary IgA levels between groups at different times, we found that the control group had significantly higher pretest salivary IgA levels at 24 weeks' GA ($p < 0.001$) and 32 weeks' GA ($p = 0.031$) than that at baseline (16 weeks' GA). Conversely, the time effects in the intervention group showed no significant between-group differences in salivary IgA levels before yoga at each data collection time *vs.* baseline, except for 36 weeks' GA ($p = 0.018$) (group × week interaction terms, Table 3; Fig. 3).

4. Discussion

Our study contributes to the literature by documenting monthly changes in healthy pregnant women's salivary cortisol and IgA levels after prenatal yoga from 16 to 36 weeks' GA. Our results show that immediately after practicing yoga pregnant women's salivary cortisol levels decreased and their salivary IgA levels increased. Yoga also had significant long-term effects on salivary IgA levels at 36 weeks' GA, compared to IgA levels in the control group. However, we found no significant long-term effect on salivary cortisol levels. These results suggest that prenatal yoga significantly benefited women by reducing their stress and strengthening their long-term immunity, supporting our hypothesis that regularly practicing yoga mitigates pregnant women's stress (lowers salivary cortisol) and enhances their immune function (increases salivary IgA).

Our finding on the immediate effects of yoga exercise on salivary cortisol levels echoes reports that prenatal yoga immediately lowers women's salivary cortisol levels.[6,27,30,31] Similarly, our findings are in line with reports that regular yoga lowered salivary cortisol levels in non-pregnant female students[54] and older women.[55] No matter the study context (western or Asia countries), the population (pregnant or non-pregnant women) or age (students or older adults), yoga effectively relieved stress.

Our trial offers evidence that a complementary therapy such as yoga helps to restore pregnant women's HPA balance and reduce their stress response, as previously reported.[6,27,30,31] However, those studies did not explore the long-term effects of yoga. Instead, saliva samples were collected before and after yoga only twice during pregnancy.[6,27,30,31] In our study, however, saliva samples were collected before and after prenatal yoga every month across pregnancy. We did not find a long-term effect of yoga on salivary cortisol levels, possibly because cortisol levels are easily affected by confounding factors such as circadian rhythms, seasonal changes, salivary flow rate, mood, sleep, and other life stressors.[6,51] Indeed, pregnant women in today's society feel great stress, and the dose and frequency of yoga may have been insufficient to generate

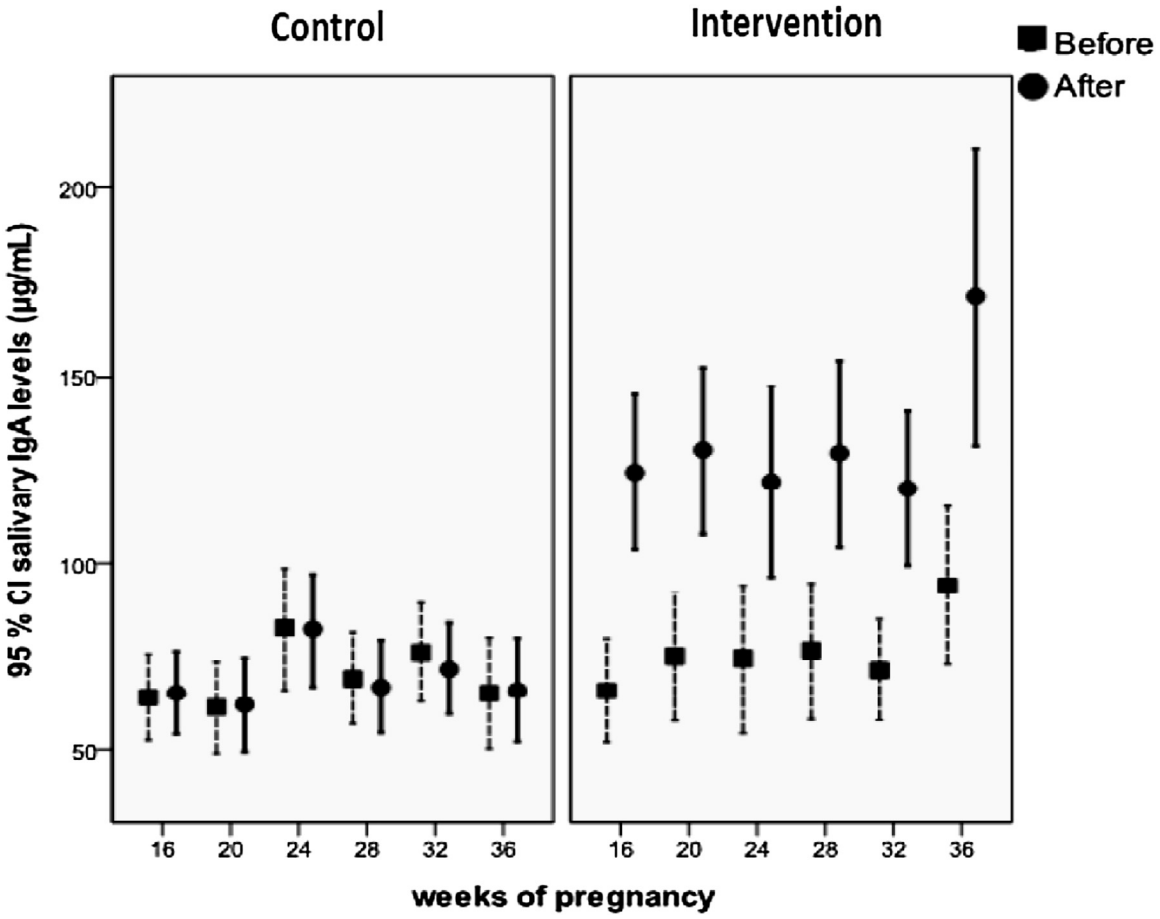

Fig. 3. Overall differences between the intervention and control groups in salivary IgA (μg/mL) levels. Changes in salivary IgA levels at six times during pregnancy for women receiving yoga (intervention group) or routine prenatal care (control group).

long-term effects. Our finding that women's salivary cortisol levels increased with increasing GA is consistent with a report that salivary cortisol levels were higher at 38–39 weeks' GA than at 30–31 weeks' GA.[56] These results can be explained by pregnancy inducing maternal adrenal glands to secrete adrenocorticotropin, which stimulates the adrenal cortex to produce cortisol, a normal regulatory and feedback mechanism during pregnancy.[11,57]

Although cortisol levels normally increase during pregnancy, excessive cortisol concentrations may suppress immune function.[34–36] Our finding of an immediate effect of yoga on pregnant women's salivary IgA levels is consistent with a report that yoga significantly increased salivary IgA in non-pregnant women after six of 10 yoga training sessions.[58] We found not only an immediate effect of yoga on IgA levels, but also a possible long-term effect. However, yoga exercise has been found to not significantly increase the immune function of older adults (mean age = 73.21 years),[59] possibly due to the declining function of older adults' immune system.[60] Further studies are needed to explore the effects of yoga on immune function in different age populations. Our finding on the long-term effects of yoga on pregnant women's immune function is new, warranting confirmation in future studies.

Yoga is considered a moderate-intensity physical activity for pregnant women.[61] We found that yoga is also a convenient and cost-effective exercise for pregnant women to manage their stress. For example, we only needed to provide yoga mats and aids and one certified yoga instructor per 10 participants. Another benefit of a prenatal pregnancy program was that participants could share their pregnancy-related experiences and feelings after practicing yoga, offering another way to manage their stress. The postures learned in yoga class could also help these women manage stress after pregnancy and throughout life.

Our findings also show that infants delivered by women who practiced prenatal yoga weighed significantly more at birth than infants delivered by women in the control group. Furthermore, women in the intervention group had a significantly longer mean GA (but ≤40.1 weeks' GA) than women in the control group. However, four women in the control group delivered infants at 36 weeks' GA, and two delivered low birth weight infants (<2500 g). These findings may be due to yoga relaxing uterine arteries and increasing blood flow to the uterus in pregnant women practicing yoga.[51] Our results are consistent with previous reports that yoga benefits pregnant women[2,29,39] and can lead to improved birth outcomes (e.g., gestational age, Apgar scores, birth weight and number of preterm births).[2]

Our study has several strengths. First, its outcome variables were biological markers of both salivary cortisol and IgA and were sensitive enough to detect the immediate and long-term effects of prenatal yoga. Second, the intervener was a midwife and certified prenatal yoga instructor. Third, the study was a randomized control trial, with acceptable study powers for both salivary cortisol and IgA. Fourth, we used the GEE method's generalized linear models with 3-way interaction, a powerful analytic method to compare group outcomes. Finally, our study is the first to follow pregnant women practicing yoga from 16 to 36 weeks' GA.

However, our study had some limitations. First, we did not measure the effects of prenatal yoga on long-term clinical outcomes and other physiological markers of immune and adrenal function (i.e., serum IgG, IgA, IgM and dehydroepiandrosterone sulfate). Second,

the dose and frequency of yoga may not have been enough to produce long-term effects on salivary cortisol levels. Third, although the outcome assessor and intervener were different, they were not completely blind to all research processes because they could clearly distinguish between the two treatment conditions. Future studies on this topic might include other physiological markers, recruit more pregnant women, and vary the yoga dose and frequency. Finally, we excluded participants who could not attend 85% of yoga sessions. Those pregnant women might not have enjoyed exercising and have had tight schedules. To overcome this selection bias, future studies could offer more a flexible yoga schedule to increase the attendance rate and reward participants who could frequently attend yoga sessions.

5. Conclusion

Prenatal yoga significantly reduced the stress hormone cortisol and enhanced the immune biomarker IgA during pregnancy. The study evidence suggests that practicing yoga positively influences pregnant women's health. Women in Taiwan, a developing country with a highly competitive society, especially need to learn how to relieve life stress. Clinicians also need to learn the mechanisms of yoga and its effects on pregnant women. Our findings can guide clinicians to help pregnant women alleviate their stress and enhance their immune function. Future studies in different countries and cultures could recruit larger samples to develop and modify yoga programs to fit pregnant women's physiological and psychological changes and examine the effects of yoga on biological and psychological outcomes.

Conflict of interest statement

All authors declare no conflicts of interest with the research.

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