Original Article

Efficacy and Safety of Parenteral Human Placental Extract and Oral Azithromycin Versus Oral Azithromycin Alone in Chronic and Recurrent Pelvic Inflammatory Disease : An Open-Label Randomized Controlled Trial in Indian Patients

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Background : Treatment of Pelvic Inflammatory Disease (PID) is challenging due to symptom recurrence with broad-spectrum antimicrobials. Placental therapy combines antimicrobial with antibiofilm actions, in addition to other benefits.

Aims and Objectives : To compare the efficacy and safety of parenteral Human Placental Extract (HPA) and Oral Azithromycin (AZ) *versus* AZ monotherapy in chronic and recurrent PID.

Materials and Methods : This prospective, multicenter, open-label study was conducted in 60 consenting eligible subjects having PID. Subjects were randomly allocated to receive either 2ml intramuscular HPA injection once daily for 2 weeks plus 1g oral AZ once daily for 1 week or only 1g oral AZ once daily for 1 week. The primary endpoint was treatment response based on the Clinical Global Impression (CGI) scale at 2 and 12 weeks. Relapse/recurrence rate at 12 weeks was a secondary endpoint. Incidence of Adverse Events (AEs) was the safety endpoint.

Results : A significantly higher proportion of subjects in HPA+AZ showed 'excellent' response at 2 weeks compared to those in AZ (75.86% *versus* 50.00%; p=0.046); this significant benefit of HPA+AZ over AZ was sustained till 12 weeks (79.31% *versus* 38.46%; p=0.002). Subjects in HPA+AZ had fewer symptom relapses than those in AZ (17.24% *versus* 23.08%; p=0.589). Improvement in PID signs and symptoms was also evident. Mild AEs in 10.00% subjects in each arm resolved by study end.

Conclusion : This study showed that subjects treated with a combination of HPA and AZ responded better compared to those treated with AZ alone with respect to symptom alleviation of chronic PID.

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***Key words* : Pelvic Inflammatory Disease, Human Placental Extract, Azithromycin, Randomized Controlled Trial.**

Pelvic Inflammatory Disease (PID) is one of the most prevalent gynecological disorders in women of reproductive age. It is an inflammatory condition in which the upper reproductive tract (endometrium, fallopian tubes, ovaries, pelvic peritoneum and adjacent pelvic structures) is infected, often by multiple ascending microbes from the lower genital tract (vagina and cervix) to the uterine cavity; lymphatic or hematogenous routes of infection are rare. Untreated PID causes severe morbidity and complications such as chronic pelvic pain and intra-abdominal infections in addition to infertility, ectopic pregnancy and preterm labor that are of global concern due to their effects on reproductive health[1-5]. These also increase the risk of psychiatric disorders leading to decrease in Quality

Editor's Comment :

- Human placental extract has antimicrobial, antibiofilm, and repair properties. Its concomitant administration with antibiotic showed benefits over antibiotic monotherapy in symptom alleviation of chronic/recurrent pelvic inflammatory disease, a prevalent gynecological disorder that may cause infertility. Relapses were also comparatively fewer with the combinatorial treatment, although study in a larger cohort is required to substantiate the same.

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of Life (QoL)[6]. The age-standardized rate of global prevalence of PID and ectopic pregnancy was 53.19 and 342.44 per 1,00,000, respectively in 2019[1-5]. The rate of association between PID and infertility was 9%-85% worldwide with a low perception prevalence[7].

PID manifests as acute, chronic, or subclinical infection; it is often underdiagnosed due to the wide variation in nature/severity of symptoms. Diagnosis is primarily based on comprehensive history and clinical and physical examinations[5,8]. Due to its polymicrobial etiology, broad-spectrum antimicrobials are commonly used for management[5]. However, a challenge is symptom recurrence despite conventional treatments[8]. Biofilms provide a protective niche to microorganisms thus enabling them to thrive in the presence of antimicrobials. Such biofilm-producing pathogens increase the risk of PID[9] and limit the success of treatment modalities.

Placental therapy has been successfully utilized in multiple indications including PID, chiefly because Human Placental Extract (HPA) exhibits antimicrobial and antibiofilm properties in addition to facilitating tissue repair/regeneration, wound healing, debridement, pain relief, immunomodulation, anti-inflammation and cellular proliferation.These properties are attributed to a composition rich in biomolecules such as Polydeoxyribonucleotide (PDRN), Nicotinamide Adenine Dinucleotide Phosphate Hydrogen (NADPH), ubiquitin-like peptide, corticotropin-releasing hormone (CRH)-like peptide, growth factors and glutamate[10-12].

Earlier studies have reported better management of PID using HPA with conventional therapy[13], antimicrobial therapy[14], or doxycycline[15,16] compared to monotherapy of these. HPA resulted in lower recurrence and prevented long-term sequelae. Compared to antimicrobials such as Azithromycin (AZ), complete remission was evident in a higher proportion of PID patients treated with HPA for 12 weeks[17]. Although HPA has long been considered as an alternative modality for PID treatment, there are only a few studies supporting its use in recurrent PID. The current study evaluated the efficacy and safety of combinatorial treatment with HPA and AZ *versus* AZ monotherapy in women having chronic and recurrent PID.

Materials and Methods

This prospective, multicenter, open-label study was conducted at four sites in India between September, 2022 and September, 2023. Patients were enrolled after obtaining written informed consent and written approvals from Ethics Committees of study sites. The study was conducted in accordance with the Indian Council for Medical Research (ICMR), New Drugs and Clinical Trials (NDCT) rules 2019 and Indian Good Clinical Practice (GCP) guidelines for clinical research (CTRI/2022/09/045480; registered on 13 September 2022).

Eligible subjects were 18- to 45-year-old women with lower abdominal/pelvic pain and cervical motion or pelvic tenderness or adnexal tenderness based on historical ultrasonography, diagnosed with recurrent PID persisting despite antibiotic treatment within past 6 weeks, having a history of infertility and willingness to receive Intramuscular (IM) injections. Post-menopausal women, pregnant/lactating mothers, participation in any other clinical trial in the past month, active treatment or evidence of active tuberculosis/ Sexually Transmitted Disease (STD), unlikelihood to comply with trial protocol,or known endometriosis or hepatic/renal impairment or any other debilitating systemic condition, which, as per the Investigator, would deem the patient unfit to participate, were exclusion criteria.

Considering 48% difference in complete symptom remission rate between treatment arms at 12 weeks[17] and accounting for 40% drop out, a sample size of 60 (30 in each arm) was computed to achieve 90% power with 5% level of significance. Subjects were randomly and equally allocated (based on a computer-generated randomization list) to receive either 2ml IM HPA injection once daily for 2 weeks plus 1g oral AZ once daily for 1 week after meals or only 1g oral AZ once daily for 1 week after meals. After treatment initiation, subjects were followed up at 1, 2 and 12 weeks (window period: ±3 days at 1 and 2 weeks; ±7 days at 12 weeks).

The primary endpoint was the number and proportion of subjects whose response was excellent, good and poor on the Clinical Global Impression (CGI) scale at 2 and 12 weeks, wherein a subject was classified as 'excellent' if majority of presenting symptoms resolved, 'good' if majority of presenting symptoms remained unresolved but adequately controlled and 'poor' if there was minimal improvement or worsening of presenting symptoms. Relapse/recurrence rate of symptoms at 12 weeks was the secondary endpoint. Safety was assessed based on incidence of Adverse Events (AEs). Subjects were also monitored for improvement in the following symptoms: pain in the lower abdomen or pelvis, abnormal vaginal discharge, dysmenorrhea, pain in the upper right abdomen, painful sexual intercourse, fever and chills, menstrual irregularity, abnormal menstrual bleeding and painful urination.

Gynecological/bimanual pelvic examination was performed for signs of PID including presence of vaginal discharge, cervical motion or pelvic tenderness, adnexal tenderness, uterine tenderness and restricted mobility of uterus.

All statistical analyses were based on the International Council for Harmonization E9 document 'Statistical Principles for Clinical Trials' and performed using SPSS version 28.0.1.1. Independent t-test was used to compare demographic characteristics and Pearson's chi-square test to compare treatment responses; $p<0.05$ was considered statistically significant.

Results

A total of 60 PID patients were screened and enrolled, with 30 in each treatment arm. Baseline demographic characteristics were comparable between the arms (Table 1). A total of 5 subjects were lost to follow-up and 55/60 (91.67%) subjects completed the study (per protocol or PP population): 29 in HPA+AZ and 26 in AZ (Fig 1). Pain in the lower abdomen or pelvis (96.36% PP subjects) was the most common symptom at baseline. Among the signs of PID, presence of vaginal discharge was the most common (87.27% PP subjects) at baseline.

On the CGI scale, a significantly higher proportion of subjects in HPA+AZ, compared to AZ, had 'excellent' response at 2 weeks (75.86% *versus* 50.00%; p=0.046). This significant treatment response of HPA+AZ over AZ was sustained till 12 weeks (79.31% *versus* 38.46%; p=0.002). On the other hand, a significantly higher proportion of subjects in AZ, compared to HPA+AZ, had 'poor' response at 12 weeks (Fig 2). Recurrence rate was lower in HPA+AZ (17.24%) than AZ (23.08%; p=0.589). By 12 weeks, all subjects showed improvement in fever and chills. Also, improvement was seen in menstrual irregularity, painful urination and presence of vaginal discharge in all subjects in HPA+AZ and in pain in the upper right abdomen, abnormal menstrual bleeding and restricted mobility of uterus in all subjects in AZ (Table 2).

Mild AEs were reported by 6/60 (10.00%) subjects (HPA+AZ, 3; AZ, 3). AEs included fever, headache, urinary tract infection, abdominal pain, lower abdominal pain and muscle inflammation. All patients with AEs recovered. No clinically or statistically significant changes were observed in body temperature and blood pressure.

Discussion

PID poses significant physical, mental and healthcare burden, particularly due to its long-term detrimental effects on reproductive health. The concerns are further amplified due to symptom recurrence[1,6,7]; possible causes, among others, include biofilm formation and incomplete infection clearance upon conventional treatments. Due to its antibiofilm properties, HPA might be used to combat such difficult-to-treat deep-seated infections[9,18].

In the current study, a significantly higher proportion of subjects had 'excellent' response to symptoms upon treatment with HPA+AZ compared to AZ monotherapy. Recurrence rate was comparatively lower with HPA+AZ. Signs and symptoms improved in concordance with earlier reports[13,14] and improvement persisted even after completion of treatment. A marked reduction in PID symptoms was reported in 27%-59% subjects treated with HPA and antimicrobial[14]. In the current study, up to 74% subjects showed improvement in individual symptoms with HPA+AZ;

Table 1 — *Demographic characteristics of subjects at baseline*

	HPA+AZ (n=30)	AZ (n=30)	p-value
Age (years) :			
Mean ± SD	32.18±7.63	33.22±5.78	0.556
Median (min, max)	33.20 (18.20, 43.82)	32.63 (23.16, 44.59)	
Height (cm) :			
Mean ± SD	151.70±6.34	152.85±8.05	0.542
Median (min, max)	151.50 (138.50, 168)	153.50 (124.96, 168)	
Weight (kg) :			
Mean ± SD	57.58±10.10	61.66±10.56	0.132
Median (min, max)	58 (38, 80)	60.95 (39.26, 85)	

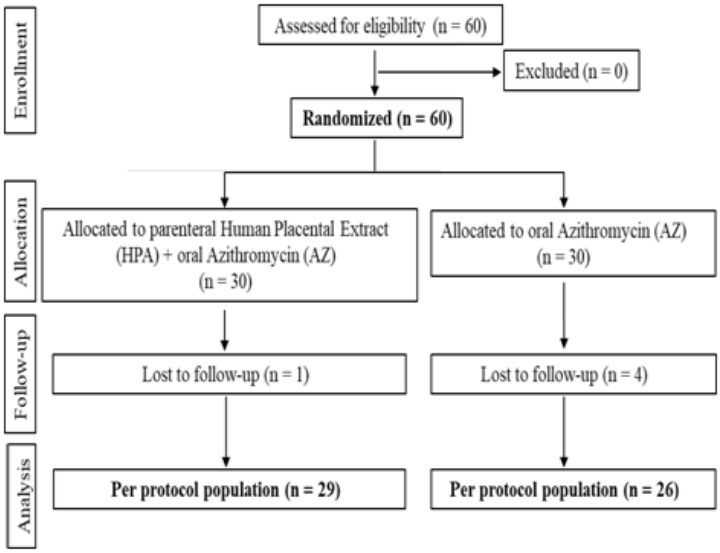

Fig 1 — Subject Disposition

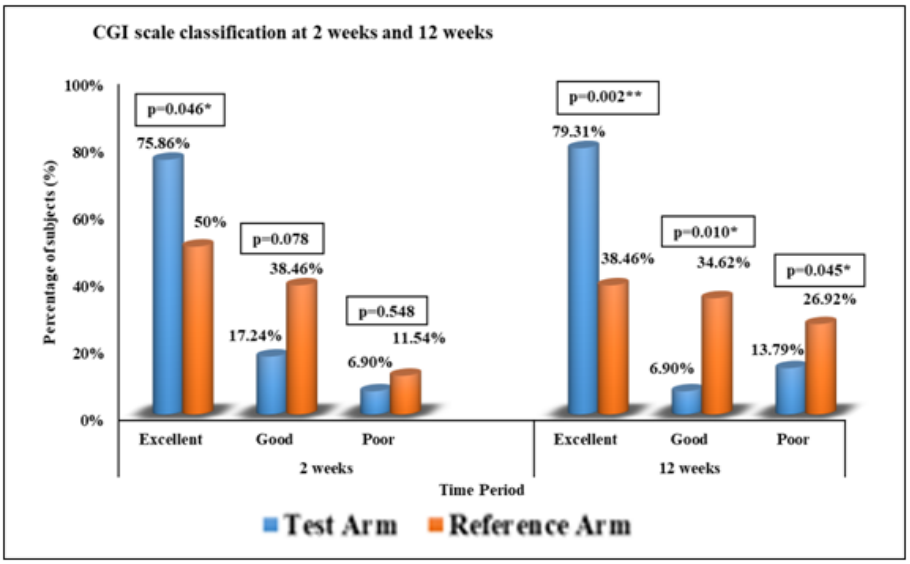

Fig 2 — Treatment response based on Clinical Global Impression (CGI) scale

improvement rates at 12 weeks were higher than those reported in an earlier study with HPA alone[17]. Notably, significant improvement was observed in the current study upon treatment with HPA+AZ for 2 weeks in patients who had been on antibiotic treatment prior to enrollment. HPA+AZ was also found to have a good safety profile. Taken together, combinatorial treatment with HPA and antimicrobial might be more effective than monotherapy, as has been reported earlier[15]. Overall, the current study corroborates existing literature on effective and safe use of this combination in PID management.

This study evaluated multiple efficacy measures including early improvement at 1 week, sustained improvement at 2 and 12 weeks and recurrence at 12 weeks. Recruitment from geographically-distributed regions across India enabled assessment of culturally diverse women from different backgrounds. However, this study is limited by its small sample size and the lack of information on causes of PID. Identification of the causative organisms in a future study would enable efficacy evaluation of HPA+AZ against specific pathogens.This is especially important because novel pathogens pose considerable challenges in PID management[8] and data on strain-specific prevalence is limited. In earlier studies, HPA was shown to cause a significant reduction in biofilm-forming ability of infectious organisms by inhibiting/decreasing bacterial motility, pyocyanin and pyoverdine, extracellular matrix of biofilm and cell surface hydrophobicity. Further studies are required to explore the efficacy of HPA+AZ in PID caused by biofilm-producing pathogens, to investigate

Table 2 — *Improvement in symptoms and signs of PID*

	HPA+AZ				AZ			
	Baseline, n	1 week, n (%)	2 weeks, n (%)	12 weeks, n (%)	Baseline, n	1 week, n (%)	2 weeks, n (%)	12 weeks, n (%)
Clinical symptoms of PID :								
Pain in the lower abdomen or pelvis	27	22 (81.48%)	23 (85.19%)	20 (74.07%)	26	22 (84.62%)	22 (84.62%)	20 (76.92%)
Abnormal vaginal discharge	26	22 (84.62%)	24 (92.31%)	24 (92.31%)	22	18 (81.82%)	19 (86.36%)	19 (86.36%)
Dysmenorrhea	22	13 (59.09%)	16 (72.73%)	19 (86.36%)	17	10 (58.82%)	10 (58.82%)	11 (64.71%)
Pain in the upper right abdomen	21	18 (85.71%)	19 (90.48%)	20 (95.24%)	17	15 (88.24%)	16 (94.12%)	17 (100.00%)
Painful sexual intercourse	9	5 (55.56%)	8 (88.89%)	8 (88.89%)	7	3 (42.86%)	4 (57.14%)	4 (57.14%)
Fever and chills	8	7 (87.5%)	8 (100.00%)	8 (100.00%)	3	1 (33.33%)	1 (33.33%)	3 (100.00%)
Abnormal menstrual bleeding	7	3 (42.86%)	5 (71.43%)	6 (85.71%)	3	1 (33.33%)	2 (66.67%)	3 (100.00%)
Menstrual irregularity	6	1 (16.67%)	6 (100.00%)	6 (100.00%)	5	0 (0%)	1 (20.00%)	3 (60.00%)
Painful urination	3	2 (66.67%)	3 (100.00%)	3 (100.00%)	4	3 (75.00%)	4 (100.00%)	3 (75.00%)
Gynaecological / Bimanual Pelvic Examination :								
Presence of vaginal discharge	25	21 (84.00%)	24 (96.00%)	25 (100.00%)	23	18 (78.26%)	19 (82.61%)	19 (82.61%)
Cervical motion or pelvic tenderness	24	18 (75.00%)	19 (79.17%)	20 (83.33%)	20	17 (85.00%)	18 (90.00%)	19 (95.00%)
Adnexal tenderness	20	15 (75.00%)	14 (70.00%)	17 (85.00%)	16	15 (93.75%)	15 (93.75%)	15 (93.75%)
Uterine tenderness	9	7 (77.78%)	8 (88.89%)	8 (88.89%)	6	4 (66.67%)	5 (83.33%)	5 (83.33%)
Restricted mobility of uterus	4	4 (100.00%)	4 (100.00%)	2 (50.00%)	1	1 (100.00%)	1 (100.00%)	1 (100.00%)

NOTE : Percentages are computed based on the number of symptomatic subjects at baseline.

possible association of the antibiofilm property of HPA with in vivo outcomes, and to identify the HPA component(s) responsible for its action[12]. A longer follow-up period in a larger cohort to assess recurrence rate and pregnancy outcome in PID patients with infertility would be useful too. Comparison between patients on different doses of antibiotic would also be worthwhile to investigate if the antibiotic dose can be lowered when co-administered with HPA.

Conclusion

This study showed that a combination of HPA and AZ is effective and safe for mitigation of signs and symptoms of chronic and recurrent PID; it results in excellent patient response at as early as 2 weeks with sustained effect and low recurrence and AE rate till 12 weeks.

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Conflict of Interest : VV is an employee of Albert David Limited. The other authors declare no conflict of interest.

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