Biochemical pregnancies: how should they be interpreted?

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ABSTRACT

There is little information in the literature about biochemical pregnancies (BPs). However, BPs (pregnancies which regress prior to imaging with ultrasound) are a major problem in IVF and recurrent miscarriage clinics. The reported incidence of between 13-22% of pregnancies may be confounded as today's sensitive pregnancy tests may detect endometrial, pituitary or phantom hCG. Additionally, a false positive result may be due to extaneous hCG administered in an ART cycle. Hence the author has suggested the presence of rising hCG levels at two consecutive tests as a definition, and that one raised hCG level should be described as isolated. The etiology remains unclear. Embryonic aneuploidy, thinned endometrium, sperm defects and defective angiogenesis have been suggested. Additionally, a number of BPs are early ectopic pregnancies which fail to develop further.

Future live birth rates of 59% have been reported after BPs; however, BPs may be recurrent. The author treats recurrent BPs as recurrent pregnancy loss. However, treatment to prevent further BPs is empiric, with no evidence in the literature. The author uses hCG supplementation to enhance implantation. The results show a non-significant improvement in the subsequent live birth rate. The author has used IVIg in patients with >5 BPs. Nine live births were achieved in 19 pregnancies (47%). However, the results may be confounded as the previous BPs may have been early ectopic pregnancies, and the subsequent pregnancy intra-uterine.

If a BP becomes persistent, and hCG levels fail to fall, methotrexate may be required as in early ectopic pregnancies.

KEYWORDS

Biochemical pregnancies, pregnancy of unknown location, non visualised pregnancy.

Definition

Biochemical pregnancy (BP) is often also known by other terms, including chemical pregnancy, non-visualized pregnancy or pregnancy of unknown location (PUL). The most common definition of a BP is a positive βhCG test with no pregnancy on ultrasound. The most recent nomenclature is that of the European Society of Human Reproduction and Embryology (ESHRE), published in 2015 [1]. The classification is based on a number of previous definitions. If there is a decreasing βhCG level and no localization of the pregnancy on ultrasound, if performed, the pregnancy is known as a non-visualized pregnancy [2]. If no ultrasound has been performed, the pregnancy loss has been called a "biochemical pregnancy" [3]. If the pregnancy resolves spontaneously after expectant management, the pregnancy is known as a resolved PUL after expectant management [4]. If medical management such as methotrexate (MTX) is used, the pregnancy loss is known as a treated pregnancy loss after medical management.

The problem with all of the above definitions is that if βhCG levels are closely monitored, as is common in *in vitro* fertilization (IVF) programs, low levels of βhCG will be picked up and interpreted as a BP. Hence, there have been more strict definitions in the past, including an initial level of 10-1000iu hCG and a subsequent rising level [5,6]: Coulam *et al.* [7] used a definition of two or more raised values of hCG with no gestational sac 3 weeks after embryo transfer.

The use of the term PUL suggests that the pregnancy may be ectopic. We suggest an alternative nomenclature: isolated raised hCG level, and biochemical or non-visualized pregnancy if the hCG level rises, when no extraneous hCG is administered.

As isolated raised hCG levels may peak and rapidly fall, the lack of substantial delay in the onset of the next menstrual period distinguishes a BP from a clinical pregnancy. The different outcome of a BP compared with a clinical pregnancy has led the American Society of Reproductive Medicine (ASRM) to distinguish BPs from clinical pregnancies, and not to recognize BPs as miscarriages. However, the European Society of Human Reproduction and Embryology (ESHRE) does recognize BPs as pregnancy losses, similar to miscarriages, partly based on Kolte *et al.*'s [2] observation that each non-visualized pregnancy loss reduces the chance of a subsequent live birth by 10% (RR, 0.90, CI 0.83; 0.97), similar to the risk conferred by each additional clinical miscarriage.

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Isolated raised hCG levels

hCG can be detected from 7 days after ovulation [8], and can be used clinically from 9 days after the LH surge. A positive hCG test after 12 days is usually taken as indicative of pregnancy. However, modern tests are so sensitive that phantom, endometrial or pituitary hCG can also be detected. A low positive hCG value does not invariably mean that trophoblastic hCG is present. The tests may be confounded. Some tests use animal antibodies raised to hCG. If the patient harbours anti-animal antibodies after exposure to the same animal used in the test, a false positive result may occur. Additionally, if hCG was used for ovulation induction, it may still be present after 12 days. van de Weijer *et al*. [9] showed low amounts of hCG as a contaminant in hMG, and Kol [10] showed hCG to be present in Corifollitrophin α. Intra- and interlaboratory variation may also lead to false positive results. These low levels of hCG are isolated raised hCGs, not biochemical pregnancies.

Incidence of biochemical pregnancies

Various authors have assessed the incidence of BPs. In fertile patients, incidences in the range of 13-22% have been reported [11-13]. Isolated raised hCG levels were reported in 4% of Liu *et al*.'s series [14]. In the infertile population the incidence has been reported to be 14%-18%, which is not higher than in the fertile population [15,16]. However, a higher incidence has been reported in IVF patients (22-31%) when compared with the general infertile population [17-19], and a rate of 22.5% was reported in Tamhankar *et al*.'s [20] study, as opposed to 5% in women who conceived spontaneously. Salumets *et al*., [15] who studied a series of 1242 frozen embryo transfer cycles, reported that increased maternal age at IVF/ICSI was the only parameter elevating the BP rate, rather than IVF itself.

Causes of biochemical pregnancies

The cause of BPs may be dependent on the embryo, the mother, or even, possibly, the father. However, there is no information on BPs and sperm abnormalities. There is only information on male factors and recurrent pregnancy loss or recurrent implantation failure.

Embryo causes

Delayed implantation may be a cause or result of pregnancy loss, as hCG may be insufficient to allow implantation. The hCG produced at the start of pregnancy is mainly the hyperglycosylated form (hCG-H) [21,22]. hCG-H is autocrine in nature, produced by the cytotrophoblast. hCG-H drives the invasiveness of the syncytiotrophoblast. In Sasak1 *et al*'s [23] study only 8 of 36 BPs produced > 40% hCG-H on the day of implantation. All (100%) normal term pregnancies produced hCG-H levels greater than 40%. Alternatively, if implantation is delayed, there may be a slow rise in hCG levels. This slow rise may indicate abnormal embryonic development. Abnormal embryonic development may have occurred after implantation either due to genetic or other embryonic factors [24].

As recurrent implantation failures and recurrent miscarriage are known to have a high incidence of aneuploidy, it is thought that BPs may be due to a genetic aberrations. However, genetic aberrations have never been shown in BPs.

Maternal causes

Endometrial thickness has been reported to impact on BPs. In Dickey *et al*.'s report [25], BPs were found in 21.9% (7 of 32) of pregnancies if the endometrial thickness was less than 9 mm on the day of hCG administration in women undergoing ovulation induction, but in none of 49 pregnancies where the endometrial lining was greater than 9 mm. Hence, it is possible that a thin endometrium may not allow proper invasion by the trophoblast, leading to inappropriate placentation. Additionally, hCG secretion by the invading trophoblast may be negatively modulated by endothelin-1 (ET-1), or PG F2α found in the endometrium [26]. Oxidative stress can also enhance hCG levels, while not leading to necrosis and apoptosis of the trophoblastic epithelium [27].

Implications of biochemical pregnancies

There is no question that the occurrence of a BP is extremely psychologically distressing for both partners. They feel joy in achieving a pregnancy after prolonged infertility, only to have that happiness dashed by pregnancy loss. Hence, the stress associated with BPs has led to patients leaving IVF programs [28].

The occurrence of a BP is a positive predictor of future IVF pregnancies [6,29], but a negative predictor of pregnancy outcomes, as BPs are associated with higher recurrent BP and miscarriage rates [30]. Each non-visualized pregnancy loss has been reported to confer a decreased risk of a subsequent live birth (RR, 0.90, CI 0.83; 0.97), equivalent to the RR conferred by each additional clinical miscarriage [2]. In cases of exclusively recurrent biochemical pregnancies, the risk of ectopic pregnancy has been reported to be 27% [31]. However, between 6% and 20% of women with a sporadic BP have an ectopic pregnancy [32].

Management

BPs may be non-viable or present with persistent raised hCG levels. In some cases, treatment with MTX may be required in order to induce trophoblast regression. MTX acts by halting DNA synthesis. In ectopic pregnancy, MTX is associated with a 67-94% success rate. However, it should be borne in mind that hCG initially rises when the trophoblast regresses. A meaningful fall in hCG is only seen after 7 days. Side effects such as stomatitis, gastrointestinal distress, dizziness, neutropenia, reversible alopecia, abdominal pain and vaginal bleeding or spotting may also be seen.

There is currently insufficient information in the literature to allow the formulation of guidelines for the management of patients after recurrent BPs. Below are some suggestions based

on the author's experience; they are not evidence based. If there is one isolated BP, it is this author's opinion that there is little need for active treatment. In the case of two consecutive BPs, there is still little need for active treatment. However, the ESHRE regards two BPs as two pregnancy losses, and therefore can be assumed to support treatment to prevent recurrence. In cases with three or more BPs, the present author treats the patients as if there were three or more miscarriages. Our database contains the details of 40 patients with two or more BPs who did not receive active treatment in the index pregnancy. There were 21 subsequent live births (53%). Eight subsequent pregnancies terminated as missed miscarriages (20%). There were ten subsequent BPs and one ectopic pregnancy.

Specific medications (author's experience)

There is little information on various drugs used to improve the live birth rate. As stated above, hCG-H accounts for 90% of the total hCG in first two to three weeks of pregnancy when invasive trophoblast activity is highest [21,22]. Hence, a luteal dose of hCG is often administered in IVF practice in order to enhance implantation. Theoretically, hCG-H may prevent pregnancy failure at the time of implantation. However, hCG-H is patented and not commercially available, therefore, generally, commercially available hCG can be used instead. hCG prevents further miscarriages in recurrent miscarriage [33]. The author has used hCG supplementation in 28 patients with three or more BPs: 20 subsequent pregnancies ended in live births (71%). However, the numbers are too small to determine whether this 71% live birth rate is significantly different to the 59% seen in the control group (10 live births in 17 pregnancies).

The author has used intravenous immunoglobulin in patients with five or more BPs. Nine live births were achieved in 19 pregnancies (47%).

Conclusions

It is clear that much more data on BPs are needed. Databases need to be combined in order increase the number of patients available for assessment. One possible source of "big data" is the database of the Society for Assisted Reproductive Technology (SART). However, as the SART does not recognize BPs as pregnancies, it would be necessary to search the database for hCG levels.

In recurrent pregnancy loss, there is a concept that the endometrium loses its selectivity, and allows abnormal embryos to implant. These abnormal embryos are then lost as miscarriages [34]. In recurrent BPs the opposite may be true, that the endometrium does not allow normal embryos to implant, and that these are lost as biochemical pregnancies.

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