MIRACLE OF ZAMZAM WATER: THE EFFECT ON HUMAN ENDOMETRIAL AQUAPORIN

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ABSTRACT

Introduction: Zamzam water is unique in its natural characteristics; zamzam water has special optical parameters that are different from those of bottled drinking and distilled water. The Aquaporin (AQP) are a family of (small 25-34 kDa) that facilitate rapid passive movement of water the aim of this work is to study the effect of zamzam water on endometrial expression of aquaporin.

Material and methods: 50 healthy fertile women divided into 2 groups, group I (n=25) intake of 500-750 c.c of zamzam water for one month, and group II (n=25) intake of tape water, at the time of insertion of intrauterine contraceptive device endometrial biopsy was taken and immunohistochemistry for detection of AQP2, AQP3, AQP4, AQP7, AQP8, AQP9, AQP10, were done.

Results: statistically significant increase in endometrial AQP2, AQP3, AQP4 in zamzam water drinking group (group I) $P<0.05$. Expression of new Aquaporin in group I AQP 7, AQP 9, AQP 10 in zamzam water drinking group.

Conclusion: One of the miracle zamzam water is expression of a new aquaporine and increase of already expressed aquaporine.

Keywords: Zamzam water, Aquaporin, endometrium, endometrial receptivity.

INTRODUCTION

Zamzam water is unique in its natural characteristics, zamzam water has special optical parameters that are different from those of bottled drinking and distilled water[(1)].

Aquaporins are water selective membrane proteins active in tissues with high water transport[(2)]. The first water channel was identified inhuman erthrocytes in 1992 (AQP1) [(3)], the AQPs are a family of small 25-34 κD4 hdyrophobic integral membrane channel protein that facilitate, rapid passive movement of water.

To date 13 Isoforms of AQPs (AQPO-QP12) have been identified in membranes based on sequence homology data. Phylogenetic comparison and permeability properties AQPs (AQP0- AQP 10) are now subdivided into two major groups[4,5]. Orthodox AQPs and Aquaglyceroporins, the group of orthodox AQPs is composed of six members these are water selective channels and permeable to water but not to small organic and inorganic molecules (AQPO, P 1, 2, 4, 5, 6).

The group of aquaglyceroporins includes four members (AQP 3, 7, 9, 10). They are non selective water channels which are permeable to glycerol, urea, and other small non electrolytes as well as to water[3]. Aquaporins exist in the plasma membranes as hemotetramers with each AQP monomer containing two hemi-pores which fold together and thereby form a water channel. The AQPs are all expressed differently and in a unique manner in tissues during development[6].

Patient and Methods

50 healthy women with no history of medical disorder, no history of drugs, hormones in the last 3 months divided into 2 groups.

Group (I): Had intake zamzam water only 500 c.c for one month.

Group (II): Had intake of tape water as a control.

After consenting, endometrial biopsies were obtained at the time of loop insertion to study the effect of zamzam intake on endometrial expression of Aquaporin.

Endometrial biopsies were obtained from 25 healthy women at the time of loop insertion after consenting no history of previous drug intake for 3 months. The endometrial biopsy sample were fixed in 4% phosphate buffered formaldehyde for 24 hours and thereafter stored in 70% ethanol until embedded, then biopsy samples were embedded in paraffin and cut into sections of 54 mm[7,8]. After than it was covered with 75ml blocking serum and incubated for 30 minutes in humid chamber[9,10,11,12]. The sections were then incubated with the primary antibodies for (AQP2, AQP4, AQP8, AQP9, AQP10, AQP7, AQP3) diluted with blocking serum over night 4^{o}C in humid chamber, the specificity of each type of AQP antibody has previously been shown using immunoblotting, the primary antibody was polyclonal raised in rabid and provided by Soren Nielsen[13].

Evaluation of staining intensity was performed by using a grading scale from 0 to 3 where 0= non staining, 1= faint staining, 2= moderate staining, and 3= intense staining, the number of stained cells was similar in all biopsies, three observers, each unaware of the identify of slides evaluated the staining intensity the average value from the three observers was calculated[7,12,13].

Data analysis:

Difference between endometrial AQP2, AQP3, AQP4, before and after zamzam water drinking were analyzed by two tailed t test, chi square test was used for comparison and difference of < 0.05 was considered significant.

RESULTS

25 healthy women with no medical and surgical disorder, and no intake of any drugs or hormones in the last three months, had intake of zamzam water only 500 – 750 c.c for one month group I and 25 women with intake of tape water group II, the group I, group II were subjected to endometrial biopsy (before insertion of intrauterine, device, detection of AQP in the human endometrial glandular epithelial cells, the results of our work were summarized in the following table.

Table (1): Staining intensity of AQP in the glandular epithelial of human endometrial cells

AQP	Group I (n=25) Zamzam drinking group	Group II (n=25) Non zamzam drinking group	P value
2	4.5±1.5	2.2±1.4	P<0.05
3	5.5±2.2	3.5±1.11	P<0.05
4	6.5±3.3	4.5±2.11	P<0.05
7	1.7±0.3	-	-
8	4.5±1.5	4.1±1.11	P>0.01
9	3.1±1.22	-	-
10	2.2±0.5	-	-

We found from Table (1) that:

- There were statistically significant increase in the AQP2, AQP3, AQP4 P<0.05 in Zamzam water group (Group I).
- Zamzam water group stimulate production of AQP7, AQP9, AQP10 as we do not demonstrate aquaporin in the sample group II (control group).
- No change in the AQP 8 between Zamzam water group (Group I) and non zamzam water group (Group II).

DISCUSSION

The first reported confirmation of AQP in the female reproductive system was achieved by isolating the complementary DNA (cDNA) encoding a water channel from a human uterus cDNA library template. The coloned cDNA had high (99.8%) homology to the 28 κDa human erythrocyte channel-forming integral membrane protein (CH1P28) water channel gene[14]. Now, at least 11 AQP isoform AQP1-AQP11 have been confirmed to be expressed in the female reproductive tract or in cells involved in assisted reproductive technology procedure. There specific expression pattern suggests that they play a role in water movement between the intraluminal, interstitial and capillary compartments.

Edema is characteristic of the endometrium during the menstrual cycle, the mammalian uterus undergo stromal odema in preparation for embryo implantation. **Richard et al. (2003)**[15] studied AQP1, P4, 5,8, during the peri-implantation period, their finding suggest that a subset of AQPs is involved in peri-implantaton fluid homeostasis.

AQPs may play an important role in reabsorption of luminal fluid and the antimesometrial positioning of the blastocyst[17]. It was found that AQP2 expressed at midsecretory phase suggesting that AQP2 might play a physiological role in the receptivities of human uterus[18].

The finding of **Richard et al., (2003)** suggest that (AQP 1, 4, 5) are significantly expressed in the peri-implantation uterus and AQP8-9, m RNA were expressed in the implanting blastocysts in a mouse pregnancy model, previous study by microarray analysis detected a decreased expression of AQPi gene in the endometrial implantation window suggesting a possible role of AQPs in implantation window[19]. It has been shown that estrogen is involved in the up regulation of AQP2 in the mouse uterus[20], in human the role of progesterone also included[17], but the continuous increase in the AQP2 during the luteal phase[7] suggests that other factors may be involved in this regulation so we can reach to the point that aquaporin in the endometrium involved in the endometrial receptivity and it should be one of the measurement of this receptivity[21,22,23]. In our work we demonstrated for the first time in the literature that zamzam water had an effect on endometrial AQP, we demonstrated that aquaporins expression increased in zamzam water group 2,3,4. This increase was statistically significant. In zamzam water group I there was stimulation of expression of other types of AQP (7, 9, 10). No change between zamzam group and non zamzam group regarding AQP8.

These data showed the effect of zamzam water in the endometrial receptivity and again to the best of our knowledge no report in the world literature delt with this aspect as the two groups of patients are fertile, this means that the newly expressed aquaporin (7,9,10) had no function related to the process of endometrial receptivity but may had other function yet not determined[24,25,26], the increase in the Aquaporin 2,3,4 means that aquaporins directly involved in the process of the endometrial receptivity and this increase need period of follow up after loop application to see the difference between zamzam and non zamzam group in an unpublished work we demonstrate that no need for aquaporin for zamzam water to work.

So, in conclusion we demonstrate for the first time in literature that zamzam water stimulates a new aquaporins and increased expression of some aquaporins we can benefit from all these facts for treatment of reproductive aberration in future and could be a source of incoming research to elucidate this aspect of zamzam water.

The effect of zamzam water is due to the special character of this water due to its peculiar of zamzam water which was discovered by radioimmunoassay, nano-technology, laser femto, crystaline electromicroscopy, specific refractive index,

number single oscillator, specific dispersing of optical parametersal assay, Abbe number of zamzam water one completely different from other types of water[1,27,28,29].

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