TOWARDS BETTER UNDERSTANDING OF AGE CHANGES DURING MENOPAUSE IN WOMEN IMPLEMENTING SYSTEMS BIOLOGY APPROACH

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ABSTRACT

Background: The menopause is defined in medical terms as the cessation of menstrual periods for 1 year secondary to estrogen deficiency. Decreasing serum estrogen levels manifest in vasomotor and urogenital symptoms during the climacteric stage of the menopausal transition. It is for the relief of these symptoms that the use of menopausal hormone therapy (HRT) is not disputed. **Objectives:** This thesis aims at determining potential changes in proteome profiles of women under the influence of hormone. We hypothesized that urine can be used as a source of proteins since it is non invasive and easy to obtain. Moreover, it provides a real time monitoring of protein changes. The fact that urine does not only contain makers that reflects kidneys' function but also other organs of the human body would also allow implementing a systems based approach for the interpretation of the study outcomes. **Subjects and methods:** In this work urinary proteomics were analyzed in urinary samples taken from 125 menopausal women under HRT. Comparing the results with those taken from 125 matched women not under HRT. **Results:** The data obtained showed significant differences between both groups. We hope these data,- after clinical validation- will help in answering the unanswered questions about use of postmenopausal HRT. **Conclusion:** proteomics may provide genetic profiles detailing age related disease predisposition and the anticipated effect of HRT.so that physicians may be confident about their recommendation and patients assured about their decision.

Keywords: menopause, biology

INTRODUCTION

The menopause is defined in medical terms as the cessation of menstrual periods for 1 year secondary to estrogen deficiency. Decreasing serum estrogen levels manifest in vasomotor and urogenital symptoms during the climacteric stage of the menopausal transition. For the relief of these symptoms the use of menopausal Hormone Replacement Therapy (HRT) is not disputed[(1)].

The Women's Health Initiative (WHI) hormone study, which was funded by the National Institute of Health, was halted after six years. Determined that the risks of hormone replacement therapy appeared to exceed the benefits[(2)].

Hormone debated has grown as previous studies showed risk reduction of heart attacks, bone fractures and Alzheimer's disease with HRT, however women that showed beneficial effects included women that began HRT during or after beginning of menopause[(3)].

The current recommendation of the American Heart Association (AHA) panel for cardiovascular disease prevention in women provides important information. Early intervention would reduce the rate of coronary heart disease .However the science supporting these clinical considerations is far from perfect. More and better research is needed[(4)].

In order to improve treatment and outcome, it is important to determine roles of aging versus hormone deficiency in age relating diseases, in the same vein, it is important to evaluate the risk markers for protective, prevention and management. Knowledge of these markers will allow rational choice of lifestyle changes, nutritional ad exercise management and the choice of hormones and other agents to be used in preparing for the second half of life for post-reproductive individuals[(5)].

This thesis aims at determining potential changes in urinary proteome profile of women under the influence of menopausal HRT. This study is a form of data collection. Clinical validation of the results may help to answer the unanswered questions about risks and benefits of menopausal HRT.

To better achieve these goals, urine samples were collected from menopausal women whereas Proteomics, Bioinformatics methods were implemented throughout the course of this investigation.

SUBJECTS AND METHODS

The practical part of this thesis was done in New York University School Of Medicine department of Obstetric and Gynecology during the period of August 2007 tell October 2008.

This thesis is part of Kronos Early Estrogen Preventive Study (KEEPS) which is a randomized placebo-controlled double-blinded prospective trial. It is a multicenter trial with eight centers around USA at which participants are entered and followed, and one coordinating center which administers the study.

Inclusion criteria:

1) Age between 42-58 years of age.
2) Menses absent for at least 6 months and no more than three years.
3) Good general health.
4) Have not used HRT in the last three months.
5) Have not had a hysterectomy.

Medications used:

0.45 mg of oral estrogen (Premarin® manufactured by Wyeth) as well as progesterone (Prometrium® manufactured by Solvay). The progesterone will be given during the first 12 days of the month.

Urine samples:

250 urine samples formed the material of the present work, samples were taken from preserved urine samples according to KEEPS project (Kronos early estrogen preventive study).

Urine Samples were grouped into two groups:

Group I: 125 urine samples of postmenopausal women not taken any hormonal treatment.

Group II: 125 urine samples from comparable group of women under HRT.

The protein concentration of the urine samples was measured by Bio-Rad Bradford total protein assay kit (Bio-Rad Laboratories Inc.) which is Regeant A with the catalog (500-0113) and reagent B catalog (500-0114) and by using the micro-plate photometer reader (multiskan plus) manufactured for Fisher Scientific catalog no 14-386-27 and contain four filters 405 nm, 450 nm, 620 nm and 750 nm.

Measurement principle:

The Multisken plus utilizes the original thermo-electron concept of vertical photometry in which the light beam passes through the whole sample .In vertical photometry , the absorption of light is proportional to the amount of the light absorbing substance in the wheel.

All samples for this study were run using a MALDI-TOF MS (Ciphergen, PBS II) platform in the proteomic facility I New York university school of medicine biochemistry department, protein detection was based on a spectrum of signals generated.

Protein identification is the performed through bioinformatics work using the website (WWW.PUBMED.COM)

RESULTS

Table (1) revealed that no significant difference can be noted between the two groups as regarding the demographic distributions.

Protein concentration is significantly increased in group I versus group II (table 2).

As regards the subcellular locations of identified proteins, there is a significant difference in the intracellular, nucleus and the unknown location between group I versus group II (table 3).

As regarding functions of the identified proteins, 16 proteomic functions are reported in the non- HRT users compared with only 6 functions in the

HRT users. Significant differences were noted in cell proliferation and unclassified function (table 4).

Table (1): Demographic distribution between group I versus group II and their significant difference

	Group I (NO HRT)	Group II (HRT)	P Value
Total	125	125	
Age	54.6±3.01	54.3±3.48	NS
Race			
White %	80 (88)	78 (87)	NS
Black %	22 (6)	26 (6)	NS
Asian	23 (6)	21 (7)	NS
Weight (Kg)	72.4± 19.4	75.6± 19.1	NS
BMI(Kg/M2)	26.9± (5.67)	24.6 ± (4.65)	NS
Height	162.3 ± 6.5	163.5 ± 6.1	NS
Baseline smoking status			
Current smokers, n (%)	16(15)	15(25)	NS
Past smokers, n (%)	40(25)	35(21)	NS
Never smokers, n (%)	69(60)	75(54)	
Age of menopause	52.3±2.1	51.1±3.01	NS
Marital status			
Married	65(55)	71(57)	NS
Widow	12(12)	10(13)	NS
Divorced	44(28)	36(27)	NS
Never married	4(5)	8(3)	NS
Parity			
Multipara	76(72)	82(79)	NS
Nulliparous	49(28)	43(21)	NS

No Significance difference in demographic distribution between the two groups

Table (2): Protein concentration mg/dl in urine sample in group I versus group II

	Group I	Group II	P-Value
Protein concentration	3.86	2.06	S

Protein concentration in significantly increased in group I versus group II

Table (3): Distribution of the identified urine proteins in group I versus group II according to their locations and their significant difference

Subcellular location	Group I	Group II	P-Value
Total	54	12	S
Membrane	12	4	NS
Cytoplasm	5	3	NS
Extracellular	4	1	NS
Intracellular	9	1	S
Unknown	10	1	S
cytoskeleton	2	1	NS
Nucleus	8	1	S
secreted	3	1	NS

A significant difference between the two groups as regarding intracellular, nucleus and unknown locations

Table (4): Distribution of the identified urine proteins in group I versus group II according to their function and their significant difference

Function	Group I	Group II	P-Value
Total	54	13	S
Immune Response	14	4	NS
Transcription	4	1	NS
Metabolism	10	4	NS
Cell Proliferation	7	1	S
Unclassified	8	2	S
Protein Synthesis	2	2	NS

A significant difference between the two groups as regarding cell proliferation and unclassified function

DISCUSSION

In this work, urinary proteomics of a group of postmenopausal women under HRT was compared with urinary proteomics of a matched control group not under any hormonal therapy.

The obtained data revealed that urinary protein concentration in the HRT users group is significantly increased compared with the non users group (3.86 versus 2.06).

Only 12 proteins could be identified in the urine of HRT users compared with 54 proteins in the urine of non users. Distributions of the identified proteins as regards molecular mass, subcellular location and protein function are tabulated.

HRT induced significant changes in urinary proteomic profiles. Urinary proteomics can differentiate between HRT users and non-users .Urinary proteomics biomarkers can represent a promising tool

to distinguish between HRT users and non users.

Gericke et al. [6] studied influence of hormone replacement therapy on proteomic pattern in serum of post menopausal women; proteomic study was able to distinguish between HRT users and non users correctly, yielding a sensitivity of 100% and specificity of 100%.

Dagmar et al.[7] studied proteomic biomarkers of peripheral blood mononuclear cells obtained from postmenopausal women undergoing an intervention with dietary supplementation with soy isoflvones extract, proteome of these postmenopausal women showed selected set of proteins responding to treatment that could be closely linked to genesis and progression of atherosclerotic processes.

Disease characterization and mapping by depicting specific protein profile can aid researches in understanding how biological processes were governed. Research pertaining to disease associated with menopause is a fertile area for further application of molecular diagnosis exploration [8].

However, this work is part KEEPS study project. Validation of proteomic biomarker needs further study. Biomarkers are validated either via their predictive association with important clinical outcome, or their agreement with diagnostic arrived by gold-standard methods [9].

CONCLUSION

Proteomics may provide genetic profiles detailing age related disease predisposition and the anticipated effect of HRT so that physicians may be confident about their recommendation and patients assured about their decision.

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