Enzyme Marker Study Of Uterine Tissue From Dysfunctional Uterine Bleeding(DUB)

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Summary: A study has been undertaken to probe into the biochemical level of enzyme markers in the tissue of uterus of dysfunctional uterine bleeding the cause of which is still debated.

The study group comprised of 38 uteri of DUB subjects as test, leiomyoma tissue from uterus as diseased control and uterine tissue from prolapse uterus as normal control in 40 cases each. Both the control and study groups were age matched.

The enzyme markers, studied were four enzymes with polymorphic loci e.g. (Phosphoglucomutase(PGM), Acid phosphatase(ACP), Esterase D (ESD) and Adenylate Kinase(AK) ; six enzymes with monomorphic loci e.g. Lactate dehydrogenase (LDH), Maleic dehydrogenase (MDH), 6-Phosphoglyceraldehydrogenase (6-PGD), Glucose-6-phosphate dehydrogenase (G-6-PD), Carbonic anhydrase (CA-I, CA-2).

It was observed that the specimens of the uterine tissue of DUB cases showed association in more numbers with the polymorphic markers, mentioned above, than those of the diseased and normal control tissues of uterus.

The contingency chi-square tests, performed for each polymorphic locus and for each organs showed that the phenotypic distributions among the study specimens were significantly different from the general population at the loci of PGM and ESD with an elevation in the frequency of allele I-I and 2-I at PGM and ESD loci respectively.

Thus an attempt has been made to find out the polymorphism of biochemical genetic markers at the organ level which may probably be able to point out the defect causing the disease, if more such markers are studied.

Introduction:

Dysfunctional uterine bleeding is an enigma which has given rise to a variety of speculations. Though several causes have been postulated starting from vascular disorders, hormonal imbalance, fault in hormonal receptors etc.(Davey ,1995), no apparent morphological abnormality could be detected in the uterus and endometrium which can pinpoint the cause of bleeding.

The findings of Blanco et al, (1964) and McAlpine et al, (1970) that a single enzyme can exist in several different forms in a single tissue is an important information and has added to our knowledge of enzyme changes in disease processes. While much of the isozymic patterns are population polymorphisms, it is possible that any associations of such patterns with disorder states of specific organs may reflect genetic predispositions.

The present study has been undertaken to probe into the biochemical changes of the tissue from DUB uterus in respect of certain enzyme markers, which have already been studied in the study population by Indian Statistical Institute, Calcutta.

Materials and Methods:

The study was undertaken in collaboration with the Anthropometry and Genetic unit of Indian Statistical Institute (ISI) Calcutta and Department of Gynecology and Obstetrics and Department of Pathology, Medical College, Calcutta.

The tissues studied were :

A) The study group (Set-I) – uterine tissues from 38 DUB cases diagnosed clinically and by imaging technique.
B) The diseased control group (Set II) - the leiomyoma tissue from 40 bleeding leiomyoma cases.
C) The normal control group (Set III) – the uterus from 40 cases of prolapse of uterus.

Both the control groups were age matched with the study group.

The patients and the control groups were selected from Hindu Bengali population only as ISI had mapped the gene frequency of healthy Hindu Bengali population in respect to the enzyme markers with red cell lysate as detailed below.

The following enzyme markers were studied :

a) Four enzymes with polymorphic loci e.g. Phosphoglucomutase (PGM), Acid phosphatase (ACP) , Esterase D(ESD), Adenylate kinase (AK).
b) Six enzymes with monomorphic loci – Lactate dehydrogenase (LDH), Maleic dehydrogenase (MDH), 6-phosphoglyceraldehydrogenase (6-PGD), Glucose-6-phosphatase (G-6-PD), Carbonic anhydrase (CA-1 and CA-2). These markers were used to compare the tissue lysate with RBC lysate standardised with the above mentioned markers to confirm that the population studied were uniform or homogenous.

In the present study the findings of the enzyme markers in the uterine tissue lysate was compared with findings of the enzyme markers present in the RBC lysate , in respect of the same above markers. Also comparison has been drawn in between the groups.

Methods:

The tissues for the enzyme marker study were collected fresh from OT, washed thoroughly with cold water and stored at 20°c until use. Tissue extract were prepared from each individual uterine tissue following the method of Coate et al, (1975). The extracts were subjected to starch gel electrophoresis for isozyme markers following the methods of Harris and Hopkinson, (1980), with the appropriate modifications to suit the local laboratory conditions.

Selection of tissues were done on histological study; uterus showing any unexpected abnormality were excluded from the study.

Observations:

Dysfunctional uterine bleeding (DUB) specimens did not show any morbid anatomical abnormality.

Specimens of leiomyoma and prolapse of uterus showed histological pictures appropriate for the condition.

The phenotype and the gene frequency of the uterine tissue in the three groups were equivocal with the monomorphic enzyme markers (LDH, MDH, 6- PGD, G-6-PD) CA 1 and 2, of red cell lysates. This indicated the uniformity of the population of the groups studied.

The polymorphic enzyme markers (PGM, ACP, ESD, AK) studies in the three groups studied showed (Table I) the following features :

a) Association with PGM, ACP, ESD and AK loci in 32,28,32,31 specimens respectively in the study group (Set –I), out of 38 specimens.
b) The diseased control group (Set II) showed association with all the above mentioned markers in 19 specimens each out of 40 such.
c) The specimens of the normal control group (Set III) showed association of 22,20,22,21 out of 40 such with PGM,ACP,ESD, and AK respectively.

Thus it was evident that the association of the specimens of DUB with the four polymorphic markers were more in numbers than the specimens of the control groups with the same markers.

To test the equality of phenotypic frequencies among the study group of the patients and the general Bengali population (Hindu), contingecy chi square tests were employed for each polymorphic locus and for each organ. It was evident (Table II) that the phenotypic distribution among the study specimens was significantly different from that in the general population at the loci of PGM and ESD. There was an elevation in the frequency of allele I-I and 2-I at both PGM and ESD loci (Table II). The cervical tissues also showed similar findings as with the uterine tissues.

Table I:

Gene frequency of the uterine wall in respect of enzyme (In numbers)

Enzyme system	Type	Uterine wall of DUB	Leiomyoma tissue	Prolapse of uterus uterineWall
Phosphogluco	1-1	15	8	9
mutase(PGM)	2-1	14	6	11
	2-2	3	5	2
	Total	32	19	22
	PGM-I	0.6874	0.5789	0.6591
	PGM-2	0.3126	0.4211	0.3405
	X^2	0.0104	2.3576	0.2790
Acid phosph	A-A	2	3	2
atase (ACP)	A-B	10	8	7
	B-B	I6	8	11
	Total	28	19	20
	p^a	0.2500	0.3684	0.2750
	p^b	0.7500	0.6316	0.7250
	x^2	0.0034	0.1722	0.2989
Esterase D	1-1	19	10	12
(ESD)	2-1	10	6	8
	2-2	3	3	2
	Total	32	19	22
	ESD-1	0.7499	0.6842	0.7273
	ESD-2	0.2501	0.3158	0.2727
	X^2	0.8884	1.3772	0.1527
Adenylate	1-1	30	18	20
	2-1	1	1	1
	2-2	0	0	0
	Total	31	19	21

Discussion:

In this study, the polymorphism of the biochemical genetic markers in respect of few only, were examined in the incised organ of a specific disease in Bengali population. No data is so far available the polymorphism at the organ level in this population.

An association between genetic markers and organ disorders would imply an identifiable genetic component in the etiology of the disorder or suggest causal relationship where the association may be due to multiple effects of some gene or to involvement of one major locus, contributing a large part of variability., combined with sufficient extragenetic, environmental and random variation .

This study has found significant association with PGM and ESD loci of DUB uterus. However, the results reported in this study can not be considered as a definitive overview of organ allele spectra in Bengali population as the study of small sample size can not account for such a large and diverse population. But this study has allowed a preliminary description of the distribution of the polymorphic alleles in this population with a specific disease and gives a new element to the database of organ

Table II.

Comparison of phenotype distribution of the study group and the general population at three loci.

Uterine wall	Genetic marker enzyme	Chi-square	
Uterine wall of DUB		Value	df
Of DUB	Phosphoglucomutase (PGM)	5.2469*	2
	Acid phosphatase (ACP)	0.4715	2
	Esterase D (ESD)	6.0648*	2
Of Leiomyoma	phosphoglucomutase (PGM)	0.9485	2
	Acid phosphatase (ACP)	1.5873	2
	Esterase D (ESD)	0.8296	2
Of Prolapse uterus	Phosphoglucomutase (PGM)	0.8945	2
	Acid phosphatase (ACP)	1.5463	2
	Esterase D (ESD)	3.5463	2

specific allele frequencies in a particular enigmatic diseased organ of DUB.

In primary DUB, it has been suggested that main abnormality of menometrorrhagia occurs at the time of menstruation and results from the disturbances of the mechanism of menstruation e.g. disturbances in the eicosanoid metabolism, in fibrinolytic system and in lysosomal enzyme systems of endometrium and uterus (Sheppard and Bonner,1983; Rybo,1966; Dockeray et al, 1987 , Bonner et al , 1983).Still lacking is the knowledge of initiating event triggering the abnormal bleeding.

Several authors (Blanco et al , 1964 ; McAlpine et al, 1970 , Coates et al, 1975) have documented that the enzyme changes can trigger the mechanism responsible for the diseased process without any apparent morphological abnormality in the target organ. It has also been postulated that a defect in the target cell enzyme gene in the form of mutation or deletion of crucial gene can ultimately precipitate the disease (Dockeray et al, 1987; Bonner et al, 1983). McDevitt and Bodmer (1974) have proposed a diseased gene akin to the 'Immune response' gene as the cause of ultimate cause of disease.

In the present study, with small number of available markers, a significant association has been observed with PGM and ESD loci in the uterine tissue i.e. the target organ. The study with more number of genetic enzyme markers will probably be able to find out more number of such genetic enzyme marker associations, triggering the disease process.

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