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Hyperphosphatemia in Infantile Hypophosphatasia: Implications for Carrier Diagnosis and Screening

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Summary

Twenty obligate carriers of infantile hypophosphatasia (HOPS), a severe autosomal recessive metabolic bone disorder, were studied and compared with 36 controls. Decreased serum alkaline phosphatase activity and increased urinary phosphoethanolamine excretion were confirmed in the HOPS carriers. Relative hyperphosphatemia was documented for the first time in the carriers. Logistic regression analysis was used to develop models for the diagnosis of and screening for HOPS carriers in the high-risk population of Manitoba Mennonites. Models based on serum alkaline phosphatase activity and on serum phosphate levels with or without urinary phosphoethanolamine excretion were used for diagnostic purposes. A model based on serum alkaline phosphatase activity and on the serum phosphate level was the most suitable for screening.

Introduction

Infantile hypophosphatasia (HOPS) is a severe, often fatal inherited metabolic bone disease. This form of hypophosphatasia, which presents within the first 6 mo of life, is generally considered an autosomal recessive disorder (Fraser 1957; Igbokwe 1985). There are milder juvenile and adult forms of hypophosphatasia; however, the genetics of these disorders and their relationship to HOPS are unclear.

The biochemical hallmarks of all forms of hypophosphatasia are decreased activity of serum alkaline phosphatase (ALP) and increased urinary excretion of phosphoethanolamine (PEA) (Eastman and Bixler 1983). Serum calcium (Ca) is normal except in infantile cases, where hypercalcemia can be seen secondary to renal failure (Rasmussen 1968). Serum phosphate (Pi) had previously been reported to be normal in patients with hypophosphatasia (Fraser 1957; Rasmussen 1968). Whyte and Rettinger (1987) recently reported hyperphosphatemia in 27 individuals affected with various forms of hypophosphatasia. Hyperphosphatemia has not been previously documented in HOPS carriers.

The present study forms part of a larger study on the genetics of hypophosphatasia and was undertaken to refine existing methods of HOPS carrier assignment and to determine whether HOPS carriers are hyperphosphatemic. Although others have shown that ALP is often decreased and that PEA is often increased in carriers, results of assays performed in different laboratories with different methodologies cannot readily be applied to our population. Also, the accuracy of these tests in carrier assignment has not been established (Eastman and Bixler 1983; Timmons et al. 1987). Note that throughout the present paper "accuracy" has been used according to a standard definition, i.e., true positive and true negative results divided by the total predictive results (Department of Clinical Epidemiology and Biostatistics, McMaster University Health Sciences Centre 1981). Results which were inconclusive were not included in our calculation of accuracy. Logistic regression analysis was used in the present study for modeling carrier assignment. Our ultimate goal is to develop a screening program designed to detect HOPS carriers

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in a high-risk population. As MacPherson et al. (1972) first noted, the Mennonite communities of Manitoba and Saskatchewan are such a high-risk population. Preliminary analysis of our data on the basis of the number of affected infants born in Manitoba among the Mennonites suggested that the prevalence of HOPS in this population is 0.5–1.3/1,000 (Chodirker 1988), which corresponds to a carrier frequency of between 1/14 and 1/24. Separate aspects of our overall study are reported elsewhere (Chodirker et al. 1987; Greenberg et al. 1989).

Methods

A. Ascertainment and Family Contact

As part of a larger study on the genetics of hypophosphatasia, multiple sources of ascertainment were used to locate probands with any clinical form of hypophosphatasia in Manitoba. For this part of our study, 20 obligate HOPS carriers (i.e., parents of 10 affected infants) and 36 controls (i.e., apparently unrelated spouses of the carriers' first-degree relatives) were studied. As there is no accepted "gold standard" test yet for defining carriers, we have made the assumptions that all parents of affected infants were carriers and that all controls were noncarriers. We recognize the limitations of this assumption (see Discussion). All individuals studied were over 18 years of age. All infantile cases were of Mennonite descent, and consequently all of the parents and most (32/36) of the controls were also Mennonite. All affected infants had typical radiologic features of HOPS, i.e., marked osseous undermineralization with rachitic-like changes most pronounced at the metaphyses. A summary of the infantile cases is shown in table 1. Four patients have previously been reported by others (MacPherson et al. 1972; McGuire et al. 1987). In all cases, the parents of an affected infant had documented low or low normal ALP.

B. Biochemical Studies

Blood samples were drawn after an overnight fast, and measurements of serum ALP, Ca, and Pi were performed on a BMC Hitachi 737 analyzer. Serum Pi was not measured if the sample was visibly hemolyzed. PEA levels were quantified from morning urine samples by a LKB Alpha Plus amino acid analyzer equipped with a high-resolution column and a lithium-based eluent system. Student's *t*-tests were used to test for statistical significance.

C. Development of Models for Carrier Diagnosis and Screening

Logistic regression analysis (Breslow and Day 1980) using the Statistical Analysis System (SAS Institute Inc 1986) was used to develop models (synonymous with equations) which could separate obligate carriers from controls. This procedure uses multiple variables to separate carriers from controls. As ALP, PEA, and Pi were found to be statistically different between the two groups univariately, the seven possible combinations of these three biochemical parameters were evaluated in the logistic regression analysis. To determine the best-fitting model, statistical comparisons of these seven models were drawn from the likelihood-ratio text (Breslow and

Table 1

Summary of Infantile Cases

Patient	Sex	Y.O.B.	A.O.P.	Age at Death	ALP
AP	M	1986	Prenatal	Stillborn	<5 U/L
RH	F	1975	1 mo[a]	9 mo	5–8 U/L
JQ[b]	M	1985	Birth	<24 h	"Absent"
CT	F	1980	Birth	6 d	12 U/L
BK[c]	M	1970	Birth	3 d	1.0 U/L
LD	F	1985	Birth	5 d	10 U/L
PF[b]	M	1984	Prenatal	<1 h	?
BT	F	1986	Prenatal	Stillborn	101 U/L[d]
AW	M	1986	Birth	1 d	5 U/L
BH[c]	F	1963	Birth	13 d	"Absent"

[a] Self-limited seizures noted on day 1.
[b] Previously reported by McGuire et al. (1987).
[c] Previously reported by MacPherson et al. (1972).
[d] ALP activity measured on a fetal blood sample at 32 wk gestation. Meconium peritonitis present.

Table 2

Results of Logistic Regression Analysis

Model	Likelihood Ratio Test: Versus Intercept G	df	P	Likelihood Ratio Test: Versus L(ALP) G	df	P	Constant	Regression Coefficients: ALP	Pi	PEA
1. L(ALP)	50.95	1	<.0001				−.316	11.743		
2. L(PEA)	21.48	1	<.0001				−4.972			.596
3. L(Pi)	18.82	1	<.0001				−10.243		7.939	
4. L(ALP + PEA)	52.66	2	<.0001	1.71	1	.19	8.446	−.292		.355
5. L(ALP + Pi)	55.79	2	<.0001	4.84	1	.03	−.664	−.322	11.398	
6. L(Pi + PEA)	30.20	2	<.0001				−13.087		6.863	.576
7. L(ALP + Pi + PEA)	55.91	3	<.0001				−1.656	−.304	10.935	.114

Note.—Carrier probability was generated from derived equation $1/(1+e^{-y})$, where y = constant + (coefficient × parameter 1) + (coefficient × parameter 2) + ... ; e.g., for L(ALP + Pi + PEA), carrier probability = $1/(1 + e^{1.656 + 0.304\,\text{ALP} - 10.935\,\text{Pi} - 0.114\,\text{PEA}})$.

Day 1980). The likelihood-ratio test is the difference of two log-likelihood statistics. This test describes the fit of each model with the highest log-likelihood χ^2 (G) reflecting the best fit. A model is considered to be predictive if the P value is <.05. The likelihood-ratio test was also used to test for the significance of adding different combinations of additional parameters to ALP. ALP was considered to be the primary factor on which to base additional models, as the χ^2 value for L(ALP) was higher than that for L(PEA) or that for L(Pi) (table 2). One model was considered significantly better than another model if the P value was <.05. By then applying an individual's biochemical results to the derived equations, a probability, i.e., a value between zero and one, was generated. Diagnostic criteria for each equation were then selected by choosing upper and lower cut-off values which would allow 100% accuracy with the fewest number of unclassified individuals. For each set of criteria, a probability below the lower cut-off would classify an individual as a noncarrier. Values above the upper cut-off would signify assigned carriers. Those individuals with intermediate values were considered not classified by those criteria.

Only individuals on whom these three biochemical results (i.e., ALP, Pi, and PEA) were available were included for this part of the analysis. This resulted in the exclusion of three obligate carriers and one control. In addition, individuals who had biochemical values that were greater than 4SD from the group mean were excluded (Anscombe 1960; Draper and Smith 1969). This resulted in the exclusion of one control, whose PEA was 6.4 SD above the control group mean.

Table 3

Comparison of Obligate Carriers and Controls

Biochemical Parameter in Each Group	N	Mean	SD	P
ALP:[a]				
Obligate carriers	20	25.7	7.5	<.0001
Controls	36	64.6	20.1	
PEA:[b]				
Obligate carriers	18	9.4	3.2	.004
Controls	35	6.2	4.6	
Pi:				
Obligate carriers	19	1.33	.17	<.0001
Controls	36	1.10	.19	
Ca:				
Obligate carriers	20	2.32	.13	.42
Controls	36	2.29	.14	

[a] Normal range 30–125 U/L.
[b] Normal range <10 umol/mmol creatinine.
[c] Normal range 0.81–1.45 mmol/L.
[d] Normal range 2.10–2.60 mmol/L.

Results

Highly statistically significant differences were found in the distributions of ALP, PEA, and Pi between obligate carriers and controls (table 3). The sex and age distributions between the two groups did not differ significantly (data not shown).

Table 2 illustrates the results of the logistic regression analysis. Although all seven models are statistically significant ($P < .0001$) and are able to separate carriers from noncarriers, the four models using ALP either alone or in combination with other parameters are comparatively better in fit (i.e., have a higher χ^2 value). Among these four models, L(ALP) is the most

Table 4

Individuals with Indeterminant Test Results

Individual	Status	L(ALP + Pi) Value	L(ALP + Pi + PEA) Value
1	Obligate carrier	.21	.31
2	Obligate carrier	.34	.42
3	Control	.22	.12
4	Control	.57	.80

parsimonious. There is no significant improvement in fit by adding PEA to L(ALP) (P = .19). However, there is significant improvement by adding Pi (P = .03). L(ALP+Pi) and L(ALP+Pi+PEA) are not statistically different (χ^2 = 0.12, P = 0.73). The probability of HOPS carrier status was derived using the constants and regression coefficients shown in table 2.

As there is no independent assessment of carrier frequency in this population available to guide our choice of cut-offs, we chose cut-offs which gave apparent 100% accuracy, i.e., obligate carriers were never called noncarriers and controls were never called carriers. Upper and lower cut-off values for L(ALP+Pi) which allowed for this apparent 100% accuracy were .2 and .55, respectively. Four individuals (two obligate carriers and two controls) were unclassified by this approach (table 4). Similar cut-off values for L(ALP+Pi+PEA) were .25 and .8. Although accuracy was still 100%, only three of these individuals (two obligate carriers and one control) were unclassified (table 4). There were no instances in which one model predicted that an individual was a carrier while the other model predicted that that individual was a noncarrier.

Discussion

Our findings concur with MacPherson et al. (1974) that there is an increased incidence of HOPS in the Mennonite population of Manitoba. On the basis of our overall study including these data, preliminary estimates of the HOPS carrier frequency in Manitoba Mennonites range from 1/14 to 1/24 (Chodirker 1988). The Mennonite community of Manitoba is a population at high risk for HOPS and as such could potentially benefit from a carrier detection program (Chodirker 1988). Using logistic regression analysis, we now report the development of accurate screening and diagnostic carrier tests for HOPS in this population.

We have made the first observation of relative hyperphosphatemia in HOPS carriers. We have confirmed that ALP is significantly lower and that urinary PEA is significantly higher in obligate carriers compared with controls (table 1). The finding of a significant difference in the serum Pi value between carriers and noncarriers is most interesting. Only recently did Whyte and Rettinger (1987) report relative hyperphosphatemia in patients with hypophosphatasia. This is now the first documentation of such a finding in carriers for the infantile version of the disease.

The Pi values for carriers and noncarriers (table 3) were mostly within the normal range for our laboratory and may explain why published reports have stated that the serum Pi is normal in carriers (Eastman and Bixler 1983; Tangney 1979; Albeggiani and Cataldo 1982). Thus, had we relied only on individual results, we would merely have stated that most were "normal." The relative hyperphosphatemia in the carriers only became apparent when the two groups were compared, and the differences were found to be highly significant.

The mechanism for this relative hyperphosphatemia is not clear. Whyte and Rettinger (1987) have postulated that increased renal reabsorption of phosphate may be the explanation. The population we have described would be an excellent one with which to test this hypothesis and to further explore the role of ALP in bone metabolism.

Carrier Diagnostic Test

A diagnostic carrier test was needed as a stringent postscreening test and also for use in other research studies. It was required that this test correctly assign the carrier status in most people. The highly significant differences in ALP, PEA, and Pi between obligate carriers and controls could now be exploited to develop such diagnostic carrier tests. Although all models based on various combinations of ALP, Pi, and PEA are statistically significant, two models were clearly the best fitting (i.e., L(ALP+Pi+PEA) and L(ALP+Pi). Therefore L(ALP+Pi) is clearly the model of choice, as it is the more parsimonious of the two. From a practical

standpoint, we have elected for future studies to use L(ALP+Pi+PEA) only when L(ALP+Pi) is inconclusive. This results in fewer individuals being excluded from a given study. For other studies on this population, we plan to use the following sequential approach for prospective carrier assignment of individuals, an approach used for carrier assignment of individuals participating in our linkage study (Greenberg et al. 1989): All individuals with L(ALP+Pi) values $\leq .2$ are called *noncarriers.* All individuals with L(ALP+Pi) values $\geq .55$ are called *carriers.* For individuals with intermediate L(ALP+Pi) values, we then used L(ALP+Pi+PEA). Those with L(ALP+Pi+PEA) values $\leq .25$ are called *noncarriers,* and those with values $\leq .8$ are called *carriers.* When this approach is used, only 6% of this study population is not classified. This approach should ideally be verified on an independent population. Cut-off values may need to be modified as experience grows. Individuals who remain unclassified after L(ALP+Pi+PEA) may benefit from newer tests, such as vitamin B, loading with measurement of pyridoxal-5′-phosphate, to ultimately determine their carrier status (Whyte et al. 1985).

Our results with this approach have shown both linkage between *HOPS* and *RH* and extremely close linkage between *HOPS* and *ALPL* (Chodirker et al. 1987; Greenberg et al. 1989). This is consistent with the results of Smith et al. (1988), who used a completely different method, i.e., CEPH families to show linkage between *ALPL* and *RH.*

We should, however, emphasize that the equation we used for the diagnosis of HOPS carriers was derived from a specific population and that results were essentially from one laboratory. We would not recommend applying the equation directly to individuals from other populations tested with different methods. We have deliberately confined our analysis to persons over 18 years of age to avoid the problems relating to different age-specific normal ranges. Consequently the equations derived do not apply to individuals younger than 18 years of age. In this younger population, DNA typing may be more valuable.

Carrier Screening Test

In contrast to a diagnostic test, a screening test has a different emphasis. In this case 100% sensitivity is essential even at the cost of overdiagnosing a few individuals initially. The individuals detected as potential carriers would then have to be tested by more definitive means. If too many individuals, however, are falsely diagnosed as carriers initially (i.e., because of too low specificity), the screening test loses its value.

We recommend the following approach to population screening for hypophosphatasia carriers in this high-risk population: ALP and Pi can be measured on a single blood specimen. If L(ALP+Pi) is $<.2$, that individual is unlikely a carrier and no further testing would be recommended. IF L(ALP+Pi) is $\geq .2$, that person should be considered a potential carrier. If L(ALP+Pi) is $\geq .55$, that individual is almost certainly a carrier (provided he or she is not affected with adult or juvenile hypophosphatasia). Further testing can be done to confirm this. If L(ALP+Pi) is $>.2$ but $<.55$, it is unclear whether that person actually is a carrier. Further testing, i.e., pyridoxal-5′-phosphate, PEA, and/or family studies, would be necessary.

Applying such an approach to our study population of obligate carriers and controls resulted in a sensitivity of 100% and a specificity of 96%. In real terms, this means that all obligate carriers and two of 33 controls were labeled as potential carriers. We have reason to believe, on the basis of linkage studies and other information, that one of these controls (individual 4; table 4) was in fact a carrier. Ideally, one would like to test the sensitivity and specificity on another population of known carriers and noncarriers. This, however, is not possible at present. The cut-off values used in an actual screening program may need to be modified somewhat as our experience grows. As one obligate carrier had an L(ALP+Pi) value very close to the cut-off, one may decide to use a cut-off less than .2. This would however, result in decreased specificity.

Major goals of this project were to develop better methods of diagnosing and screening for HOPS carriers. We believe that the finding of relative hyperphosphatemia in these carriers is an important step toward attaining these goals. Given that the Mennonite communities of Manitoba and Saskatchewan are a population at high risk for HOPS, we feel that a screening program designed to detect HOPS carriers would be valuable. Couples both of whom are found to be HOPS carriers could then receive genetic counseling. Prenatal diagnosis is one option available to these couples. Thus, this screening program would be analogous to the screening programs designed to detect Tay-Sachs carriers in Ashkenazi Jews.

Other factors would, however, need consideration before such a HOPS screening program could be developed or implemented. At present the inheritance patterns of juvenile and adult hypophosphatasia are not

clear, not is it understood how these forms of hypophosphatasia relate to HOPS. A screening program may, however, detect adult patients or juvenile carriers and inappropriately classify them as potential HOPS carriers. As the incidence of all forms of hypophosphatasia is high in the Mennonite population (MacPherson et al. 1972; Chodirker 1988), implementation of a screening program for HOPS carriers would be very problematic until the overall inheritance of hypophosphatasia is clearly elucidated. Segregation analysis and molecular studies in our population will address this problem.

We should also emphasize that, as with the diagnostic model, this model for screening only applies to this population studied in our laboratory. In addition to scientific issues, many ethical issues remain. We need to investigate how the Mennonite community in Manitoba would view a screening program designed to detect carriers of a currently untreatable disease. Obviously, before such a program could be developed, the input and cooperation of this target population would be crucial.

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