TECHNICAL NOTE

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Tests of Genetic Markers on Aborted Fetal Material

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ABSTRACT: Women who conceive as a result of rape often elect to abort the fetus. We describe twelve cases where genetic markers were tested on the aborted fetal material to provide evidence of the genetic constitution of the rapist. Two cases are presented in detail, and the problems encountered with the testing are discussed.

KEYWORDS: pathology and biology, genetic typing, criminal sex offenses

In a small proportion of rape cases, an unusual type of evidence is available to link the crime with the perpetrator. If the rape victim conceives as a result of the sexual assault, genes from the rapist become part of the genetic makeup of the resulting fetus. In such cases, genetic testing in the form of modern parentage determination may be performed to provide evidence for prosecution for rape. This type of testing is most conclusive with infants over six months of age. However, in many cases of conception after rape, the woman elects to terminate the pregnancy by abortion. This does not mean that the genetic evidence must be lost since the tissue recovered during the abortion still may be examined for genetic markers linking the biological father to the fetus. Although reports of parentage testing on aborted material are relatively rare in the forensic literature [1] (most reported cases of prenatal genetic testing deal with human lymphocyte antigens [HLA] testing using amniotic cell samples or chorionic villus material [2-6]), this type of testing is possible and often can produce compelling evidence.

Materials and Methods

We have examined tissue from 12 abortuses using HLA testing, red cell or red cell enzyme testing, or some combination of these. The fetal material ranged in gestational age from 8 to 24 weeks (3 were 7 to 9 weeks, 4 were 10 to 12 weeks, 1 was 16 weeks, and 3 were 19 to 24

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weeks of age). All the pregnancies resulted from rape. Most abortions were performed by suction techniques, although some of the older fetuses were delivered by prostaglandin or saline procedures. As noted in Table 1, the tissue used in the test was generally either fetal blood or cells teased from discrete fetal organs or limb muscles. When tissue teasing was necessary, the technologist would gently abrade the material with tweezers and forceps in Hanks solution or saline. The material was then strained through layers of gauze to remove tissue clumps. In two of the cases, cells were grown in tissue culture to expand the sample size. All genetic test procedures were performed using standard techniques [7-9] adapted for small volumes. In a few cases, a positive test for fetal hemoglobin was used as a marker of fetal tissue.

Results

Our experience with fetal testing is presented in Table 1. Red cell enzyme testing was successful in all cases where it was attempted. Red cell antigen testing succeeded in all cases but one (Case 5), in which too few cells could be retrieved. Six of the twelve cases produced HLA typing that defined at least the cross-reactive group of the biological father. Four of the six gave specific results, and two of these four were tried in the court system. These two cases are presented in detail in Tables 2 and 3 (Cases 1 and 6 from Table 1). Table 2 presents results from a case using heart blood as the fetal sample. The results are indistinguishable from those obtained from a six-month-old infant. We were able to exclude Father A, but not B. Father C (who was not tested by us), a close relative of B, also could not be excluded. The court case resulted in dismissal when genetic testing could not distinguish between these two men. The woman in the case was mentally incompetent and could not testify.

Table 3 presents results from a case involving a very young fetus. The abortion method used resulted in the delivery of an intact fetus providing us with more fetal tissue than was usually the case. We were not able to determine the subtype of HLA-B12 in the fetus, but other markers were clearly defined. Even though the mother and fetus were identical for each genetic system tested, we were convinced that the tissue tested was truly fetal in origin, a conclusion drawn from the anatomical location of the tissue used for testing. This case resulted in a conviction.

Case	Fetal Age, Weeks	Race	Number AF Tested	Fetal Tissue Tested	Tests With Data
1	19	w	2	blood	HLA, RBC
2	12	В	0	blood, body"	none
3	16	В	1	blood, body	RBC, ENZ
4	10	w	0	blood, body	RBC
			0	blood, body	ENZ
			1	spleen	RBC, HLA, ENZ
			1	blood	RBC, HLA, ENZ
8	24	В	15	amniotic	HLA $(X-R)^b$
				cells, blood	RBC, ENZ
9	9	w	1	body	none
10	10	В	1	cultured cells	HLA (X-R)
11	10	w	1	cultured cells, body	ENZ
12	16	В	0	body, heart	HLA, ENZ ^c

TABLE 1-Tests of fetal tissue to obtain genetic evidence in rape trials.

"Body refers to fetal tissue (usually limb muscle) teased apart.

 b HLA (X-R) = Data was obtained for the HLA cross-reactive group, but not the HLA specific antigen.

'Tests were performed with a mixture of maternal and fetal blood.

	Alleged Fathers				
Antigen System	- A ^a	В	С	Mother	Child 19-week
ABO	0	A	A	0	A
MNSs	MSs	Ns	MNSs	MNs	MNs
Rh	-ce	-ce	-ce	DCe	DCce
Kidd	JK ^{a+b}	JK ^{a-b+}	JK ^{a-b+}	JK ^{a+b+}	JK ^{a+b+}
Duffy	Fy ^{a+b+}	Fy ^{a-b+}	Fy ^{a+b+}	Fy ^{a-b+}	Fy ^{a-b+}
HLA	A2,3	A24,26	A24,26	A24,31	A24,26
	B7,w41	B 7	B 7	B7,17	B 17

TABLE 2—Blood types in case with three alleged fathers and 19-week fetus.

"Alleged Father A was excluded by ABO and HLA systems.

Discussion

Our results show that it is definitely possible to test fetal tissue for genetic markers. However, our experience also deliniates some of the many technical problems which can be encountered. The sample size and tissue type as well as the time elapsed post-abortion are critical points in determining success or failure. Also, each of the test procedures used have both positive and negative aspects.

The type and size of the fetal tissue sample obtained is primarily controlled by fetal size (generally expressed as gestational age) and by the method of abortion employed. An optimal sample consists of fetal blood obtained by heart puncture, a procedure which is usually possible only in older fetuses. Heart puncture samples are also more readily obtained if the tissue is delivered intact. Such a sample, even if it is only 1 mL in volume, may be treated in the same fashion as a paternity test performed on a newborn infant. Problems will be encountered only in regard to antigen development and maternal immunoglobulin. Lymphocytes and red cells are easily harvested, and there are no questions concerning tissue origin.

In the absence of a fetal blood sample, discrete fetal organs such as the spleen can be used. Another source of tissue could be cells teased from the muscles of the limbs. Red cells can be recovered or stains prepared from clots, but the origin of such material (that is, fetal or

Antigen System	Alleged Father	Mother	Fetus (8 weeks)
ABO	0	0	0
Rh	Dce	DCce	DCce
MN	Ν	Ν	Ν
HLA	A2	A2	A2
	B12(45),35	B12(44),16	B12 ^a ,16
	Cw4		
PGM	1+1+	2-1+	2 - 1 +
AK	1	1	1
6-PGD	Α	Α	Α
ADA	1	1	_
EAP	BA	В	В
Hp	n.d. ^b	n.d.	n.d.
EsD	1	1	1
рер А	2-1	1	1
ĊĂ	1	1	1

TABLE 3-Blood types in case with 8-week fetus.

"HLA-B12 Subgroup (B44 or B45) could not be determined.

 b n.d. = not determined.

maternal) is always suspect. HLA testing using cells from teased muscle almost invariably is complicated by high background levels of killed cells, making antigen definition extremely difficult. Red cell, stain samples, or both are often mixtures of maternal and fetal cells. (This can often be demonstrated with hemoglobin testing.) These mixed mother-fetus stain preparations often yield valuable information, but analysis must be approached cautiously. Unfortunately, most very young fetus are aborted using suction procedures which generally do not produce intact material or material whose origin is easily defined. For these reasons we have concluded that testing fetuses younger than ten weeks has too high a failure rate to be useful. Of course, exceptions to this rule are possible, as exemplified by the data presented in Table 3.

Because of the very specific and detailed genetic information obtained from tests of HLA system antigens, our initial efforts in each case focused on getting tissue appropriate to that procedure. The crucial need for living tissue to attempt HLA testing means delivering the tissue to the testing laboratory as soon as possible (within hours) after the abortion. Tissues should be transported in saline or tissue culture media and at no time should be treated with preservative. Using red cell antigens or red cell enzyme systems does not impose such demanding time constraints on sample collection, but the genetic data obtained by these tests is not so precise as HLA typing.

Although we did succeed in obtaining specific results in HLA testing (Cases 1, 6, 7, and 12), in others we obtained only information as to the cross-reacting group possessed by the biological father. This type of information drastically lowers the discriminatory power of the test, but still allows one to draw conclusions as to inclusion and exclusion. Cross-reacting groups in the HLA system are well defined [10].

The experimental nature of the procedure and possible lack of results need to be understood by all parties. However, the chance of successfully obtaining evidence where otherwise there would be none is certainly high enough to at least consider fetal testing as an option when dealing with cases involving conception following rape. Obviously, evidence gathering in the form of parentage determination through tests of a living infant is much more conclusive than that obtained from fetal testing. However, if the rape victim decides to abort, genetic testing of fetal tissue is now a possible option in case preparation.

Technical variations of HLA testing, such as two-color fluorescence [δ], which are already used in a few laboratories and the acceptance of deoxyribonucleic acid (DNA) testing for parentage determination and other forensic science purposes [11] may make the process of prenatal paternity testing easier and more exact. Care should be taken however, to use only genetic marker systems which are well developed during fetal life. The Gm and Km markers on the immunoglobulin molecule, for example, are not acceptable because of the maternal origin of most infant immunoglobulin during the early months of life.

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