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The Indian Journal of Genetics and Molecular Research

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Original Article

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Volume 1 Number 2, July - December 2012

46,XY Female: SRY and AR Basis: Genotype & Phenotype Correlation

Amudha S.*, MSc; Sayee Rajangam, Rema Devi*, Preetha Tilak***

Abstract

46,XY females are referred with different terms and The Consensus Statement on Management of Intersex Disorders (2006) presented a widely accepted system of nomenclature and proposed the umbrella term of Disorders of Sex Development (DSDs). The rationale of the present study, is to correlate the phenotype in cytogenetically confirmed 46,XY females specifically with the molecular genetic basis of SRY and AR genes and also with the karyotypes and the age at referral.

Material and Methods: Gather data from division of Human Genetics, St. John's Medical College, Bangalore. The data includes both cytogenetic and molecular genetics analysis of 46,XY females.

Results: The classified features of the probands under 12 groups were further subdivided and then, percentage occurrence of the features was calculated as per the presence or absence of SRY gene or the mutation in AR gene versus the karyotype and age at referral. Among the total features, probands for the AR gene analysis have manifested 116 (51.3%) and in them, the probands with AR- manifested 72 (62%). The absence of the uterus was the selective feature for the AR- or AR + status in 8/8 and 4/5 probands and also between AR and SRY. On the contrary, the sparse axillary hair was the feature between SRY- and SRY+ status. Probands with 46,XY karyotype have manifested 193 features (85.4%) out of which 64 were seen in AR- probands (33.3%). In AR- and with 46,XY karyotype, the absence of the uterus was noticed in the 7 probands. The features that were common in all the 4 SRY- and 5 SRY+ with 46,XY karyotype were the smooth skin, female voice, primary amenorrhea and female genitalia; but in the 4 SRY- they were the sparse axillary and pubic hair.

Discussion: In the present study, it could be interpreted that with the help of genetic counseling and appropriate management and therapy, the probands with SRY+ and AR+ could be reared as male and female individuals. It is seen that the probands have manifested age related features. It is stated that, as per the presence of the uterus and other mullerian derivatives, the phenotype of adult 46,XY females could be grouped into 3 major categories.

Conclusion: 46,XY females comprise a heterogenous group, which differ not only in their diagnostic category and anatomy but also in their journey through life to adult services. Medical and surgical care required.

Keywords: XY females; Gonadal dysgenesis; Androgen insensitivity .

Introduction

46,XY females are referred with different terms and The Consensus Statement on Management of Intersex Disorders (2006) presented a widely accepted system of nomenclature and proposed the umbrella term of Disorders of Sex Development (DSDs). In table 1 is given the new nomenclature to DSDs and in table 2 the genetic background to the conditions with DSDs. (Berra *et al* 2010)[1]

The authors also stated that as per the presence of the uterus and other Mullerian derivatives; clinically the phenotype of 46,XY females could be grouped under 3 major categories. (Table 3)

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Table 1: New nomenclature: DSDs (Source- Lee *et al* 2006)[2]

Previous	Current
Intersex XY sex reversal	DSD 46,XY gonadal dysgenesis (GD)
Male pseudohermaphroditism Undervirilised XY male	46,XY DSD
Female pseudohermaphroditism Masculinised of XX female	46,XX DSD
True pseudohermaphroditism XX male, XX sex reversal	Ovotesticular DSD 46,XX testicular DSD

Table 2: Genetic basis: DSDs (Source- Mendonca et al 2009)[3]

Groups of conditions	Clinical syndromes	Genes
Abnormalities of gonadal development	GD-Swyer's syndrome Denys-Drash syndrome Campomelic dysplasia Testicular regression syndrome	SRY,DHH,NR5A1 WT1 SOX9 -
Defects of testosterone synthesis	Leydig cell hypoplasia-LH receptor defects Steroidogenic enzyme deficiency -Lipoid adrenal hyperplasia -3 β hydrosysteroid dehydrogenase type II deficiency -17 α hydroxylase & 17,20 lyase deficiency -17 β hydrosysteroid dehydrogenase deficiency(17 β -HSD) Altered steroidogenesis due to disrupted electron transfer -P450 oxidoreductase deficiency	LHGCR -STAR,CYP11A1 -HSD3B2 -CYP17A1 -HSD17B3 PDR
Defect of testosterone processing	5 α reductase type 2 deficiency (5AR)	SRD5A2
Defects in androgen action	CAIS (Complete androgen insensitivity syndrome) PAIS (Partial androgen insensitivity syndrome)	AR AR
Ovotesticular 46, XY DSD	-	-

Table 3

1. 46, XY females with functioning testis producing AMH (antimullerian hormone) are born without the uterus. In early gestation, AMH secreted from Sertoli cells causes the differentiation of the mullerian duct system. Included are the females affected by AIS, 5AR and 17 β-HSD deficiencies.
2. 46,XY females without functioning testis and with GD do not produce AMH; thereby the mullerian duct system differentiates to form the uterus. Moreover, in the absence of the testosterone, the mesonephric ducts fail to develop & the undifferentiated urogenital sinus & external genitalia mature into the female structures. Included in majority are the females with 46, XY GD or Swyer' syndrome.
3. 46, XY women with ovotesticular DSD have variable testicular functions, which result in unpredictable secretion of AMH and variable uterine appearance. For example, hemi-uterus may develop if testicular tissue is predominantly unilateral.

In general, in the females referred with DSDs, along with the phenotype, the 46,XY status is confirmed with the cytogenetic analysis; but, the molecular basis is determined only in a small percentage of cases with DSDs (Achermann *et al* 2008)[4].

The rationale of the present study, is to

correlate the phenotype in cytogenetically confirmed 46,XY females specifically with the molecular genetic basis of SRY and AR genes and also with the karyotypes and the age at referral.

Material & Methods

At Division of Human Genetics, St. John's Medical College, Bangalore, during the period

Table 4: 46, XY female: SRY & AR basis versus Phenotype

Features	AR- (n8)	AR+ (n5)	AR (n13)	SRY- (n5)	SRY+ (n7)	SRY (n12)	AR & SRY (n25)
1.Short stature	-	-	-	-	1	1	1
2. Skin							
Smooth	6	4	10	5	7	12	22
Hirsuitism	1	-	1	-	-	-	1
Coarse	1	-	1	-	-	-	1
Hyperpigmentation	-	1	1	-	-	-	1
3. Voice							
Female	6	4	10	5	7	12	22
Male	2	-	2	-	-	-	2
Infantile	-	1	1	-	-	-	1
4. Barrel Chest	1	-	1	-	-	-	1
5. Breast							
Not Developed	6	2	8	1	1	2	10
Normal	1	2	3	-	2	2	5
Hypoplasia	1	1	2	4	4	8	10
6.Axillary Hair Growth							
Normal	1	-	1	-	-	-	1
Sparse	7	4	11	5	4	9	20
Absent	-	1	1	-	3	3	4
7.Pubic Hair Growth							
Normal	2	1	3	-	1	1	4
Sparse	5	3	8	5	6	11	19
Absent	1	1	2	-	-	-	2
8. Android Pelvis	2	-	2	-	-	-	2
9.Primary Amenorrhea	5	4	9	5	6	11	20
10. Genitalia							
Female	6	4	10	5	7	12	22
Ambiguous Genitalia	1	1	2	1	-	1	3
Hypogonadism	1	-	1	1	-	1	2
Total	56	34	90	37	49	86	176
11. Gonads							
Testis	4	3	7	-	-	-	7
Inguinal swelling	2	1	3	-	-	-	3
Ovary Streak	-	-	-	2	2	4	4
Ovary	-	-	-	-	1	1	1
Absent	2	1	3	3	4	7	10
12. Uterus							
Absent	8	4	12	-	1	1	13
Infantile	-	1	1	-	-	-	1
Present	-	-	-	1	-	1	1
Rudimentary	-	-	-	1	1	2	2
Hypoplasia	-	-	-	3	4	7	7
Antiverted	-	-	-	-	1	1	1
Total	16	10	26	10	14	24	50
Grand Total	72 62%	44 38%	116 51.3%	47 42.7%	63 57.3%	110 48.7%	226 -

Table 5: SRY and AR basis: Phenotype versus Karyotype

Features	AR+ XY (n5)	AR- (XY) (n7)	AR- (X/XY) (n1)	SRY- XY (n4)	SRY- X/XY (n1)	SRY+ XY (n5)	SRY+ X/XY (n2)	Total
1.Short stature	-	-	-	-	-	-	1	1
2. Skin								
Smooth	4	5	1	4	1	5	2	22
Hirsutism	-	1	-	-	-	-	-	1
Coarse	-	1	-	-	-	-	-	1
Hyperpigmentation	1	-	-	-	-	-	-	1
3. Voice								
Female	4	5	1	4	1	5	2	22
Male	-	2	-	-	-	-	-	2
Infant	1	-	-	-	-	-	-	1
4.Barrel Chest	-	1	-	-	-	-	-	1
5. Breast								
Not developed	2	5	1	1	-	1	-	10
Normal	2	1	-	-	-	1	1	5
Hypoplasia	1	1	-	3	1	3	1	10
6.Axillary hair growth								
Normal	-	1	-	-	-	-	-	1
Sparse	4	6	1	4	1	3	1	20
Absent	1	-	-	-	-	2	1	4
7.Pubic hair growth								
Normal	1	2	-	-	-	1	-	4
Sparse	3	4	1	4	1	4	2	19
Absent	1	1	-	-	-	-	-	2
8. Android pelvis	-	2	-	-	-	-	-	2
9. Primary amenorrhea	4	5	-	4	1	5	1	20
10.Genitalia								
Female	4	5	1	4	1	5	2	22
Ambiguous genitalia	1	1	-	1	-	-	-	2
Hypoplasia	-	1	-	1	-	-	-	1
Total	34	50	6	30	7	35	14	176
11. Gonads								
Testis	3	4	-	-	-	-	-	7
Inguinal swellings	1	2	-	-	-	-	-	3
Ovary-streak	-	-	-	1	1	1	1	4
Ovary	-	-	-	-	-	1	-	1
Absent	1	1	1	3	-	3	1	10
12. Uterus								
Absent	4	7	1	-	-	1	-	13
Infantile	1	-	-	-	-	-	-	1
Present	-	-	-	1	-	-	-	1
Rudimentary	-	-	-	1	-	1	-	2
Hypoplasia	-	-	-	2	1	4	-	7
Anti verted	-	-	-	-	-	1	-	1
Total	10	14	2	8	2	12	2	50
Grand Total: 46,XY (n21) 45,X/46,XY (n4)	44(24.3%)+64(33.3%)+38(19.7%)+47(24.3%)= 193(85.4%) 8(24.2%)+9927.3%)+16(48.5%)= 33(14.6%)							
Total	44 22.7%	64 33.3%	8 24.2%	38 19.7%	9 27.3%	47 24.3%	16 48.5%	

Table 6: SRY and AR basis: Phenotype: Age at referral: Below (<) and Above (>)14 years

Features	AR- (n2) >14	AR- (n6) <14	AR+ (n1) >14	AR+ (n4) <14	SRY- (n5) >14	SRY- (n3) >14	SRY+ (n4) <14
1.Short stature	-	-	-	-	-	1	-
2. Skin							
Smooth	1	5	1	3	5	3	4
Hirsuitism	-	1	-	-	-	-	-
Coarse	1	-	-	-	-	-	-
Hyper pigmentation	-	-	-	1	-	-	-
3. Voice							
Female	1	5	-	4	5	3	4
Male	1	1	-	-	-	-	-
Infant	-	-	1	-	-	-	-
4.Barrel chest	1	-	-	-	-	-	-
5. Breast							
Not developed	2	4	1	1	1	-	1
Normal	-	2	-	2	-	1	1
Hypoplasia	-	1	-	1	4	2	2
6.Axillary hair growth							
Normal	-	1	-	-	-	-	-
Sparse	2	5	1	3	5	1	3
Absent	-	-	-	1	-	-	3
7.Pubic hair growth							
Normal	-	2	1	-	-	-	1
Sparse	2	3	-	3	5	3	3
Absent	-	1	-	1	-	-	-
8. Android pelvis	1	1	-	-	-	-	-
9. Primary amenorrhea	-	5	-	4	5	2	4
9.Genitalia							
Female	1	5	-	4	5	3	4
Ambiguous genitalia	-	1	1	-	-	-	-
Hypogonadism	1	-	-	-	-	-	-
Total	14	43	7	28	35	19	30=176
10. Gonads							
Testis	2	2	-	3	-	-	-
Inguinal swelling	-	2	1	-	-	-	-
Ovarian streak	-	-	-	-	2	1	1
Ovary	-	-	-	-	-	-	1
Absent	-	2	-	1	3	2	2
11. Uterus							
Absent	2	6	1	3	-	1	-
Infantile	-	-	-	1	-	-	-
Present	-	-	-	-	1	-	-
Rudimentary	-	-	-	-	1	1	-
Hypoplasia	-	-	-	-	3	1	3
Antiverted	-	-	-	-	-	-	1
Total	4	12	2	8	10	6	8=50
Grand total	18	55	9	36	45	25	38=226
<14 years	55(42.6%)+36(28%)+38(29.4%)=129(57%)						
<14 years	18(18.5%)+9(9.3%)+45(46.4%)+2525.8%)=97(94.3%)						

of 35 years, from 1976 to 2010, 108 female probands were cytogenetically confirmed to have XY status. The gathered information was duly filled in the proforma. Among the 108 cases, the details were complete in 90 for the primary and secondary sexual features; out of which 12 cases with GD and 13 with AIS and the family with due consent volunteered for the genetic analysis on SRY and AR genes. Their age ranged from 4 to 39 years. The steps involved in the molecular genetic analysis were: DNA isolation, DNA quantification, PCR amplification, gel electrophoresis, sequencing PCR, direct DNA sequencing and DNA analysis and CAG repeat analysis with gene scan. (Thangaraj *et al* 2002b, 2003b, Singh *et al* 2006)[5,6,7]

Results

The classified features of the probands under 12 groups were further subdivided and then, percentage occurrence of the features were calculated as per the presence or absence of SRY gene or the mutation in AR gene (Table 4) versus the karyotype (Table 5) and age at referral (Table 6).

Among the total of 226 features, probands for the AR gene analysis have manifested 116 (51.3%) and in them, the probands with AR- manifested 72 (62%). The absence of the uterus was the selective feature for the AR- or AR + status in 8/8 and 4/5 probands and also between AR and SRY. On the contrary, the sparse axillary hair was the feature between SRY- and SRY+ status.

Probands with 46, XY karyotype have manifested 193 features (85.4%) out of which 64 were seen in AR- probands (33.3%). In AR- and with 46, XY karyotype, the absence of the uterus was noticed in the 7 probands. The features that were common in all the 4 SRY- and 5 SRY+ with 46, XY karyotype were the smooth skin, female voice, primary amenorrhea and female genitalia; but in the 4 SRY- they were the sparse axillary and pubic hair.

Observed features

Below 14 years, in AR- (n6), it was absence of uterus; in AR+ (n4) primary amenorrhea, female voice and genitalia and in SRY+ (n4) primary amenorrhea, smooth skin, female voice and genitalia. Above 14 years, in AR- (2) non-developed breast, sparse axillary and pubic hair growth, presence of testis and absence of uterus; in AR+ (n1) smooth skin, sparse axillary hair, normal pubic hair, ambiguous genitalia and inguinal swelling and in SRY- (n5) primary amenorrhea, smooth skin, female voice, sparse axillary and pubic hair, female genitalia.

Discussion

A vast literature is available on 46,XY females, AIS and SRY gene mutations. TFS includes males who may manifest female phenotype in the presence of intact SRY. In TFS, the target cells of the testosterone have deletion in the AR gene resulting in the absence of the male sexual differentiation and phenotypically female. In pure gonadal dysgenesis, the associated features are the absence of the testicular and male differentiation; testosterone and secondary sexual development. (Gardner *et al* 2008)[8]

The genesis of the 46,XY females are because of the cross over error between X and Y resulting in the transmission of SRY gene to X, in males, during the meiotic gametogenesis. Hence, the individuals with Y but without the SRY gene would become XY females with gonadal streaks rather than ovaries and poorly developed secondary sexual characters (Jorde *et al* 2010).[9]

Individuals with AIS have female external genitalia; undergo breast development during puberty; primary amenorrhea; inguinal testis; scanty secondary sexual hair; absent uterus and fallopian tube and blind vagina. It may be noted that inguinal hernia which is uncommon in girls is present, especially

bilaterally then AIS should be considered. Individuals with incomplete or partial androgen insensitivity undergo variable virilisation. Affected individuals are sterile and may have female sexual orientation. They also need the removal of the testis because of the increased risk of developing testicular malignancy and should placed on oestrogen therapy for the development of the secondary sexual characters as well as for the prevention of the osteoporosis in the longer term. (Turnpenny and Ellard 2012)[10]

From the available vast literature, the present study is discussed with the relevant publications. In the present study, from tables

Table 7

Categories	Berra et al 2010	Present study 2011
1	Without uterus: 46,XY females with functioning testis and antimullerian hormone (AMH) & without differentiated mullerian duct system. Examples: Androgen Insensitivity Syndrome (AIS) 5α- reductase deficiency (5αR) 17β-hydroxysteroid dehydrogenase deficiency (17-HSD)	Without uterus: 13/25 (52%) 13= AR-8;AR+5;SRY-5;SRY+7 Karyotypes & AR & SRY genes: 46,XY & AR- =7 45,X/46,XY & AR- =1 46,XY & AR+ = 5 46,XY & SRY- =4 45,X/46,XY & SRY- =1 46,XY & SRY+= 5 45,X/46,XY& SRY+ = 2 - -
2	With uterus: 46,XY females without functioning testis and AMH; with differentiated mullerian duct system; undifferentiated urogenital sinus and female external genitalia. Examples: 46,XY gonadal dysgenesis (GD) or Swyer's syndrome	With uterus: 12/25 (48%) 12=AR+1;SRY-5;SRY+6 Infantile uterus 1=AR+ Uterus 1+SRY- Rudimentary uterus 2= SRY-1; SRY+1 Hypoplasia uterus 7=SRY-3;SRY+4 Antiverted uterus 1=SRY+ Karyotypes & AR & SRY genes: 46,XY & AR+ = 1 (infantile) 46,XY & SRY- = 1 (present) 46,XY & SRY- = 1 (rudimentary) 46,XY & SRY+ = 1 (rudimentary) 46,XY & SRY- = 1 (hypoplasia) 45,X/46,XY & SRY- = 1 (hypoplasia) 46,XY & SRY+ = 3 (hypoplasia) 45,X/46,XY & SRY + = 1 (hypoplasia) 46,XY & SRY+ = 1 (antiverted) Female external genitalia: 22/25 AR-6;AR+4;SRY-5;SRY+7 Karyotypes & AR & SRY genes: 46,XY & AR- = 5 45,X/46,XY & AR- = 1 46,XY & AR+ = 4 46,XY & SRY- = 4 45,X/46,XY & SRY- = 1 46,XY & SRY+ = 5 45,X/46,XY & SRY- = 2
3	46, XY females with ovotesticular DSD and with variable testicular tissue, AMH function and uterus.	-

1 and 2, it could be interpreted that with the help of genetic counseling and appropriate management and therapy, the probands with SRY+ and AR+ could be reared as male and female individuals. From table 3, it is seen that the probands have manifested age related features.

It is stated that, as per the presence of the uterus and other mullerian derivatives, the phenotype of adult 46,XY females could be grouped into 3 major categories (Berra *et al* 2010).[1] In table 7 is shown the features of the 46,XY female under the 3 categories of the present study with that of the observations from the literature.

It is seen, that in the present study, based on the presence or the absence of the uterus along with the female external genitalia, 13 probands could be included into the category of AIS without uterus and 12 as GD with uterus and female external genitalia. The grouping is in accordance to the classification by Berra *et al* (2010).[1] Among the 13 AIS, 12 are under the subcategory of the study on AR gene and among the 12 GD cases, 11 are under the study of SRY gene category. From the classified presence of uterus, it is seen, that the hypoplasia and rudimentary uterus are associated to the SRY- and SRY+. The association to the karyotype and the AR gene showed the absence of the uterus in 46, XY with AR- in 7 and AR+ in 4.

The age of the presentation has led to 6 groupings:

i. Diagnosis in utero;
ii. AG at birth;
iii. Cloacal exstrophy;
iv. Inguinal hernia;
v. Virilisation at puberty;
vi. PA. (Berra *et al* 2010)[1]

In the present study, the probands fitting into the 1st and the 3rd groups have not been observed.

AG at birth

AG is considered to be the common presentation inn the pediatric age group. The presence of the Y may initiate a degree of virilisation at birth; thereby implicates the presence of the functioning testes and AMH and the likelihood of the absence of the uterus.

In the present study, the observed AG in 2 cases are associated to the absence of the uterus; 46,XY karyotype and one each with AR- and AR+ gene. One case has been referred below 14 (AR-) years and the other one above 14 (AR+).

Inguinal hernia

From literature, it is seen that the descent of the testes are androgen dependent; hence their presence indicates CAIS. (8) In a study on 93 females with CAIS 32 (34%) had inguinal hernia and their age ranged from one month to 11 years. It is also estimated that 0.8 to 2.4% of the premenstrual girls with inguinal hernia have CAIS. (9) In the present study, 3 cases of the AIS (3/13, 23% or 3/25, 12%) had bilateral inguinal hernia along with the absence of uterus and 46,XY karyotype and AR- (2) and AR+ (1) genes. 2 with AR- gene are above 14 and one with AR+ is below 14 years.

Virilisation at puberty

The features include failure in the development of the female secondary sexual characters, enlarged clitoris, deepening of the voice and excessive body hair in a male pattern. The origin of the androgens is likely to be testicular with concomitant AMH secretion and absent uterus. The diagnosis could be 5AR or 17β–HSD deficiency. In the present study, 2 with AR- and below and above 14 years and one with AR+ above 14 years manifested virilisation (coarse/ hairy/ hyperpigmented skin).

Primary amenorrhea

46,XY females presenting with PA vary in the age of their first assessment. They may not have androgen as in 46,XY GD or completely resistant to the effect of the androgen as in

CAIS. The former group is also oestrogen deficient and therefore present with pubertal delay. Women with CAIS usually have normal breast development and the presentation may be a little later than those with GD. The assessment of the uterus in women with oestrogen deficiency presenting with PA is particularly difficult with the ultrasound often reporting an absent uterus. From the experience it is suggested that it is better to delay making any conclusion regarding uterine development until at least 6 months of oestrogen priming have taken place. In the present study, PA as the chief complaint was present in 20 (AR-5/ AR+4/ SRY-5/ SRY+6). The 11 PA with SRY- and SRY+ genes are exactly matched the 11 with uterus (SRY-5/ SRY-6); the 9 PA cases with AR- and AR+ genes are associated to the 12 without uterus (AR-8/AR+4). As informed in the literature it is these cases which need the follow up for the presence of the uterus.

Specific molecular diagnosis has been made only in a small percentage of DSD cases. Instead, most diagnoses are made on clinical grounds. Among the 46,XY females, only 47.8% have had accurate diagnosis. Ideally the diagnosis should be made at birth to assure the correct multi disciplinary assessment throughout childhood. The delayed recognition could lead to greater difficulties in accepting the diagnosis. (Berra *et al* 2010)[1] For correct diagnosis, several aspects need to be considered and one of them is the hormonal assay when gonads are in situ; because after gonadectomy, it becomes difficult to make the accurate diagnosis. Genetic diagnosis should be made as early as possible. In the present study, from the clinically and the cytogenetically confirmed 108 46,XY cases, 25 volunteered for the molecular investigation.

Based on the mullerian derivatives, the 25 were referred for molecular confirmation of AR in 13 and SRY in 12. The AR mutation was determined in 5/13 (38.5%) or 5/25 (20%) and absence of SRY in 5/12 (41.7%) or 5/25 (20%) and the total is around 40% (10/25).

The age of presentation vary according to the diagnostic category and are described under 3 groups. For females with CAIS, the younger age groups comprise those who were found in utero and those presented with inguinal hernias. The second diagnostic group presents later with PA. It is interesting that women with GD are presented years after the manifestation of the delayed puberty which should be evident by age 16. In general, between the 3 groups as per the age at referral, female with AG under the label of PAIS are presented the earliest of the 3 groups. (Berra *et al* 2010) In the present study, 3 cases with AG belonged to the 1st group. PA cases were 20; out of which belonged to the 2nd and the rest to the 3rd group.

3 with AG are in the younger age group; 2 with AR- gene above 14 and one with AR+ below 14 years. Among the 20 with PA, 2 2 with SRY+ below 14 and the rest 18 above 14 years; AR- and AR+ above 14 are 5 and 4; SRY- and SRY+ above 14 are 5 and 4.

Conclusion

46,XY females comprise a heterogenous group, which differ not only in their diagnostic category and anatomy but also in their journey through life to adult services. Medical and surgical care required. A multi disciplinary team for the care and liaison with support groups.

References

1. Berra M, Lih-Mei Liao LM, Creightona SM, Conwaya GS. Review- Long-term health issues of women with XY karyotype. *Maturitas.* 2010; 65: 172 -178.
2. Lee PA, Houk CP, Ahmed SF, Hughes IA. International Consensus Conference on Intersex organized by the Lawson Wilkins Pediatric Endocrine Society and the European Society for Pediatric Endocrinilogy. Consensus statement on management of intersex disorders. International Consensus Conference on Intersex. *Pediatrics*. 2006; 118: 488-500.
3. Mendonca BB, Domenice S, Arnhold IJ, Costa

EM. 46, XY disorders of sex development (DSD). *Clinical Endocrinol (Oxf).* 2009; 70: 173-187.

4. Achermann JC, Hughes IA. Disorders of sex development. In: Kronenberg HM, Melmed S, Polonsky KS, Reed Larsen P, editors. Williams Textbook of Endocrinology. 11th ed. Philadelphia: Elsevier; 2008.
5. Thangaraj K, Joshi MB, Reddy AG, Gupta NJ, Chakravarty B, Singh L. CAG repeat expansion in the androgen receptor gene is not associated with male infertility in Indian populations. *J Androl. 2002b;* 23(6): 815-818.
6. Thangaraj K, Singh L, Reddy AG, Rao VR, Underhill PA, Pierson M, Frame IG, Hagelbe RE. Genetic affinities of the Andaman Islanders, a vanishing human population. *Curr Biol.* 2003b; 13: 86-93.
7. Singh R, Vutukuri R, Nalini JG, Baidyanath C, Lalji S, Thangaraj, K. SRY-negative 46, XX male with normal genitals, complete masculinization and infertility. *Mol Hum Rep.* 2006; 12;5: 341-346.
8. Gardner AT, Howell RT, Davies T. Human Genetics. New Delhi: Viva Books Private Ltd; 2008.
9. Jorde LB, Carey JC, Bamshad MJ. Medical Genetics. 4th edition. Philadelphia, USA: Mosby, Elsevier; 2010.
10. Turnpenny P, Ellard S. Emery's Elements of Medical Genetics. 2012. 14th edition. Philadelphia, USA: Churchill Livingstone Elsevier.

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Application of Monoclonal Antibodies in Oral Cancer

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Abstract

Monoclonal anti-CD66e antibodies developed by standard methods belonging to IgG 2 subclass were applied to formalin fixed paraffin embedded tissue of oral squamous cell carcinomas, reactivity correlated with degree of differentiation , both in intensity and percentage of cells stained.

Keywords: Oral squamous cell carcinoma; Monoclonal antibodies; Immunohistochemistry; Peroxidase anti peroxidase.

Introduction

Monoclonal antibodies against CD66e have been thoroughly studied in colorectal the initial works of Gold and Freedman, its role has been studied over decades various other carcinomas, for example gastric, esophageal, medullary carcinoma of thyroid, breast carcinoma, however its role in oral carcinoma is limited.[1] Immunoperoxidase technique using peroxidase antiperoxidase immunohistochemical purpose is increasingly being used as a tool to determine whether the tumor is primary or secondary carcinoma.[2] Tsutsumi *et al* showed staining pattern varies in frozen sections and paraffin embedded tissues and antigens were more readily retrieved in frozen sections, however other studies have shown that CEA in tissue blocks remain preserved as long as ten years.[3] Allum, Stokes, Macdonald showed that monoclonal antibodies react with tonsillar mucosa, which they said was due to cross reactivity.[4]

R.B. Pai, S.B. Pai, Lalitha R.M., showed that stage 2 or more carcinomas of oral cavity stained for anti-CEA antibodies, more so in areas of necrosis.[5]

Material and Methods

11 tissue blocks of oral squamous cell carcinoma as old as 5 years were stained for CEA.

recent cases of colorectal carcinoma were used as positive controls and two sections of normal tissue were used as negative control (Table 1).

Methods

Protocol for Staining

Primary antibody used was Anti-carcinoembryonic antigen monoclonal, class IgG2 .. (BioGenex, Anti-CEA).

1. Sectioning – 3μ m thick tissue sections are taken on salinized slides.
2. Fixation – either 4-6 hours on hot plate at 50-60 ^{0}C or overnight at 37 ^{0}C.
3. Deparaffinisation – Xylene, 2 changes of 5-10 minutes each.
4. Hydration – Graded alcohol (100%,70%,50%), 5 minutes each.
5. Wash in distilled water – two washes of

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Table 1

Tissue	Number of cases stained	Reactivity	Percentage reactivity
Normal tissue	2	Negative	0-5%
Inflamed tissue	4	Negative(3 out of 4)	0-5%/<15%
Colorectal carcinoma	2	Positive	90-100%
Well differentiated squamous cell carcinoma	3	Positive	75-100%
	1	positive	50-90%
Moderately differentiated squamous cell carcinoma.	3	Positive	50-90%
	1	Negative	<5%
Poorly differentiated SCC.	3	Negative	0-5%

5 minutes each.

6. Endogenous peroxide block- (H2O2 1.5 ml + methanol 50 ml) for 30 minutes, to remove background staining.
7. Two washes in distilled water for 5 minutes, each.
8. Incubation with background snipper for 15 minutes at room temperature.
9. Wash in distilled water – 2 changes of 5 minutes each
10. Antigen retrieval- wash with citrate buffer, incubate at 900C for 90 minutes.(Activates).
11. After cooling, wash with Tris buffer solution (TBS)—3 washes of 5 minutes,each.
12. Primary antibody – incubate with primary mouse monoclonal antibody against carcinoemryonic antigen at 40 ^{0}C, overnight in a humidified chamber.
13. Secondary antibody (biotinylated) - Incubate for 15 minutes at room temperature, in humidified chamber.
14. Wash with TBS (pH 7.4) – 3 washes of 5 minutes each.
15. Enzyme conjugate – incubate with streptavidin for 20 minutes at room temperature in a humidified chamber.
16. Wash with TBS (pH 7.4) – 3 washes of 5 minutes each.
17. Chromogen-– incubate with peroxidase substrate solution (Diaminobenzidine) for 1-2 mins.
18. Wash in distilled water 2 changes of 5 minutes each.
19. Counter stain with hematoxylin—30 seconds.
20. Wash in distilled water – 2 changes of 5 minutes each.
21. After drying, mount the slides with DPX.

Results

Tissue section were examined using light microscopy to determine the number of cells showing a positive reaction. Sections were classified as negative if less than ten percent, cells were positive if 10-50 percent cells were positive it was said to be 1+, 50-75% as 2+ 75-90% as over the intensity of staining was taken into consideration, sections showing 5-10% cells reactive with marked intensity were included as 1+, however less than 5% cells of any reactivity were taken as negative (Table 1, Fig 3,4).

All sections of normal tissue showed

Figure 1: Positive controlled –colorectal carcinoma

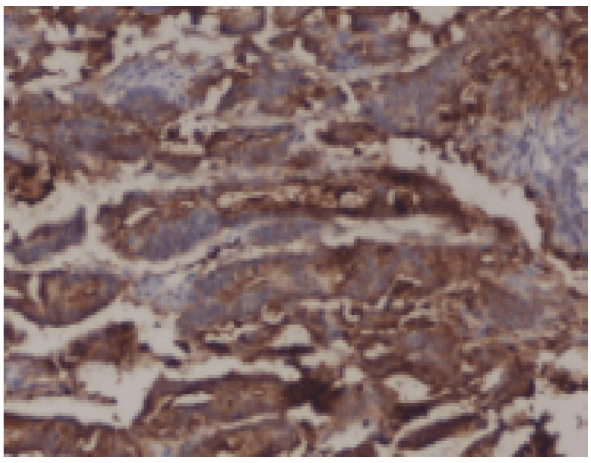
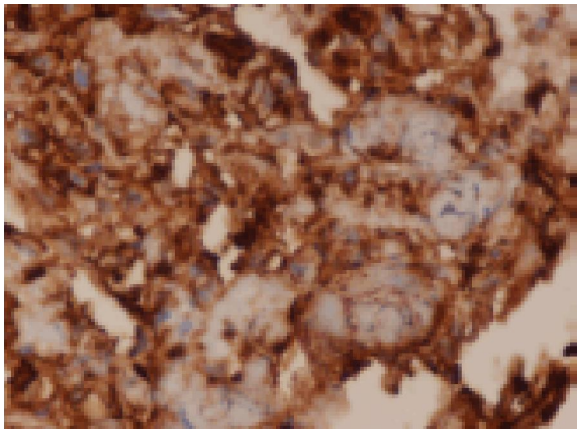


Figure 2: Well differentiated squamous cell carcinoma



negative results for monoclonal antibodies. In majority of cases inflamed tissue did not stain, however one-fourth reacted scantily. Depending upon the differentiation of carcinoma staining varied in intensity, localization and proportion of cells staining.

Well differentiated squamous cell carcinomas gave a muddy brown intracytoplasmic and membranous staining, in moderately differentiated carcinomas greater than fifty percent cells were stained, however staining was both cloudy granular and membranous, as well as golden brown intracytoplasmic (Fig 2, 3). Poorly differentiated squamous cell carcinoma did not stain, or stained with a proportion of less than 5% with membranous pattern (Fig 4). Epithelial pearls stained as eddies with paler

Figure 3: Moderately differentiated squmous cell carcinoma

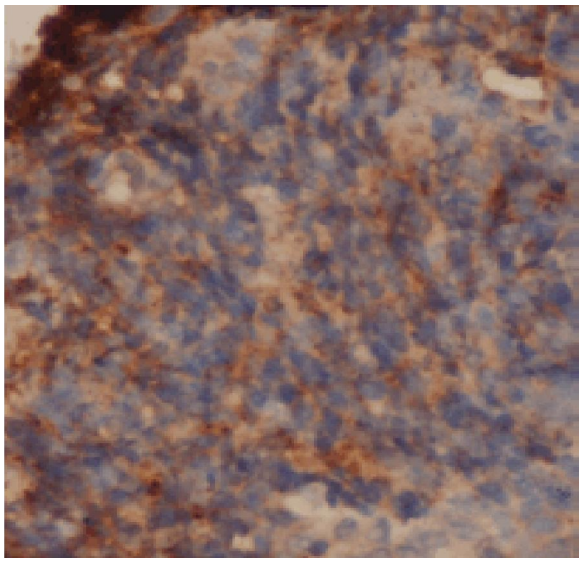
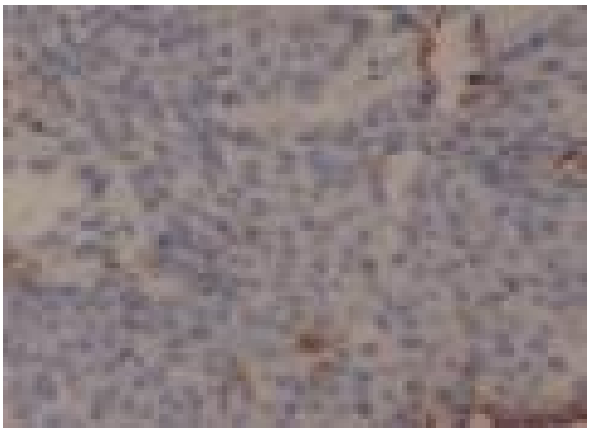


Figure 4: Poorly differentiated squamous cell carcinoma



staining as compared to the rest of malignant tissue (Fig 2). Thus comparative study of percentage cells, together with pattern indicated that carcinomas with largest number of positive cells were most differentiated.

CD66e (CEA), as a marker, has a limited role in oral cancer since usual victim of oral carcinoma is a smoker, which leads to false positive serum elevations, however tissue levels are being studied these days. Since Phil Gold and Freedman, CEA has been used as a diagnostic as well as prognostic tool in carcinomas of gastrointestinal tract; however only recently it has been used in oral squamous cell carcinoma.[6,7] Goldenberg *et al* suggested that the identification by immuno-histochemistry of tumors that express CEA could be used to determine which tumors should be monitored, however as mentioned afore that is not applicable to oral carcinoma.[8] Tsutsumi *et al* for the first time showed that staining pattern varies in frozen sections and paraffin embedded tissue, however further studies confirmed that only old specimens of paraffin embedded tissue show much variations, to obviate such confounding factor in study we used tissue sections not older than five years.[3] This study has shown the moderate to high rate of CEA expression and a direct relationship to degree of differentiation, however false positive activity in inflamed tissue may be attributed to cross reactivity with CD66a present in granulocytes, thus based on the localization of false positive can be categorized separately. Pai S.B., Pai R.B., Lalitha R.M., *et al*. showed

that oral squamous cell carcinoma express CEA with a relationship to stage of carcinoma, and staining was intense in areas of necrosis, however we did not see much variation with stage, although necrosed areas stained deeply.[5]

Conclusion

Evaluation of monoclonal antibodies using peroxidase anti peroxidase complex method employing IgG2 2 antibodies show a direct relationship with degree of histological differentiation of oral squamous cell carcinoma, albeit poorly differentiated carcinoma, which either did not react or reacted scantily.

References

1. Phil Gold, Joseph Shuster, Samuel Freedman. Carcinoembryonic antigen in clinical medicine. Historical perspectives, pitfalls and projections. *Cancer,* 1978; 42(4): 204-214.
2. Goldenberg DM, Kim EE, Delard FH, Bennet S, Primus. Radio-immunodetection of radioactive antibodies to CEA. *Cancer Res.* 1980; 40: 2984-92.
3. Tsutsumi Y, Nagura H, Watanabe K. Immunohistochemical observations of carcinoembryonic antigen (CEA) and CEA-related substances in normal and neoplastic pancreas. Pitfalls and caveats in CEA immunohistochemistry. *Am J Clin Pathol*. 1984; 82(5): 535-42.
4. WH Allum, HJ Stokes, F Macdonald, JWL Fielding. Demonstration of carcinoembryonic antigen (CEA) expression in normal, chronically inflamed, and malignant pancreatic tissue by immunohistochemistry. *J Clin Pathol.* 1986; 39: 610-614.
5. Pai SB, Pai RB, Lalitha RM, *et al*. Expression of oncofoetal marker carcinoembryonic antigen oral cancers in South India — a pilot study. *Int J Oral Maxillofac Surg.* 2006; 35: 746–9.
6. Zamchek N, Moore TL, Dhar P, Kupchik H. Immunological diagnosis and prognosis of human digestive tract cancer: carcinoembryonic antigen. *New englan journal medicine.* 1972; 286: 83-86.
7. Denk H, Tappeiner G, Echstorfer R, Holzner J H. Carcinoembryonic antigen in gastrointestinal and extra- gastrointestinal tumors and its relation to tumor cell differentiation. *Int j cancer.* 1972; 10:262-72.
8. Goldenberg DM, Sharkey RM, Primus F J. Immunocytochemical detection of CEA in conventional histological specimens. *Cancer.* 1978; 42: 1546-53.

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Wilson's Disease (Coomb Negative Hemolytic Anaemia in Acute Liver Failure)

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Abstract

Wilson's disease or hepatolenticular degeneration is an autosomal recessive genetic disorder in which copper accumulates in tissues; manifesting as neurological or psychiatric symptoms and liver disease. This has varied range of manifestations like asymptomatic hepatomegaly, subacute or chronic hepatitis, acute hepatic failure etc. Because of this there is delay in diagnosis and initiation of treatment are common, even in patients with positive family history. There is no consensus regarding therapeutic protocols since the use of penicillamine, once a 'gold standard' for treatment, has been debated by experts. Mortality and morbidity of this potentially treatable disease and nonavailability of medications to the poor patients remain a major area of concern.

Keywords: Wilson's disease; Acute hepatic failure; K.F. ring; Coomb negative hemolytic anaemia.

Introduction

Wilson's disease (hepatolenticular degeneration) is an autosomal recessesive disorder, associated with degenerative changes in brain, liver disease and Kayser-Fleischer ring the cornea. The incidence is 1/50,000 to 1/ 1,00.000 births. Girls are 3 times more likely than boys to present with acute hepatic failure. After the age of 20 yrs neurological symptoms are predominant. Wilson's disease is progressive and potentially fatal if untreated.[1]

Case Summary

A 6.5 yrs girl child, product of non consangously wedded, Para 2 couple, with normal past history was brought for complaints of yellowness of eyes without any associated complaints. On examination, vitals and general examination revealed normal except icterus and 2 cm hepatomegaly. Investigations were done at 12 o'clock which were HB-8.6 gm%, W.B.C.-17,200/cmm, N.-78%, L.-18%, S. Bilirubin-T-6.1 mg/dl,D.-4.8mg/dl. Child was admitted along with treatment of Iv fluids, inj. Ceftriaxone and antimalarials (PS-MP-negative) At 4.00 O'clock, USG abdomen done which revealed Acute liver parenchymal disease with minimal fluids in abdominal cavity. So again in the night blood investigations repeated, which was Hb-4.9gm%, TLC-31,600/cmm, L-7300/ cmm, N-21800/cmm, HCT-13.9%, RBC Count-1.62mil/cmm, Platelet count-Normal, S.Bilirubin-T-26.49mg/dl, D-18.95mg/dl, SGPT-234U/ML, SGOT-162, HBSAG-Negative, Blood gr. O positive, PT-29.09 Sec, PBS-S/O-Haemolysis. Immediately child was given blood transfusion.

On second day morning child was stable without any new complaints but her blood parameters were HB-7.3gm%, TLC-18800/ cmm, N-13000/cmm, L-4700/cmm, Platelets -1,73,00/cmm, PT-26.40 sec, G6PD Test-Negative, S.Bilirubin-T-32.5mg%,D-26.8mg%, SGPT-382U/ML, USG Abdomen was repeated-showed Acute on chronic liver disease. On same day evening blood parameters worsend, with development of

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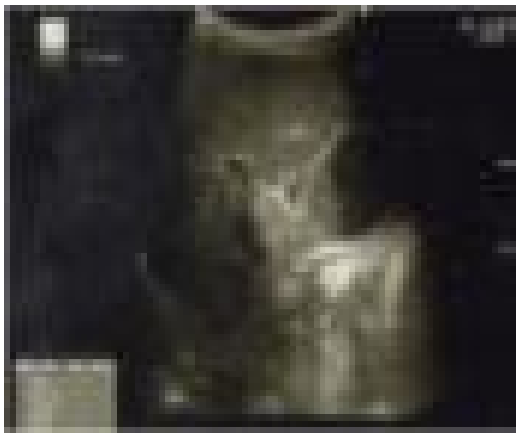
USG-Liver (2nd Day)



ascitis. Again blood transfusion and anti hepatic failure treatment started.

On third day child was comfortable with stable vitals except ascitis and slight irritability. Keeping in mind, the possibility of Wilson's disease, she was put on D-penicillamine and investigations started, Ophthalmic examination K.F. Ring on slit lamp examination, Ceruloplasmin level-12 mg%(N-20-40 mg%), raised ammonia level, negative coombs test, Negative Haemoglobinuria, deranged RFT and LFT parameters, In the evening at 4 O'clock child had altered sensorium, distension of abdomen and neck stiffness with breathing difficulty. So she was put on ventilator but within 8 hrs she died of cardio-respiratory arrest. Because of time shortage 24 hrs urinary copper analysis was not possible but after death liver biopsy confirmed the diagnosis.

Final Diagnosis

Samuel Alexander Kinnier Wilson

Wilson's Disease: Comb's negative hemolytic anaemia in acute liver failure.

Discussion

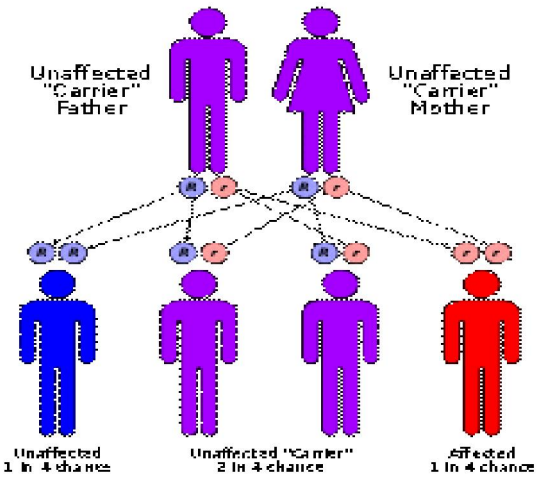
History

Wilson's disease is named after Samuel Alexander Kinnier Wilson (1878–1937), the British neurologist who first described the condition in 1912.[2]

Wilson's work had been predated by reports from German neurologist Carl Westphal (in 1883), who termed it "pseudo sclerosis"; by the British neurologist William Gower's (in 1888); and by Adolph (in 1898), who noted hepatic cirrhosis. Neuropathologist John Nathaniel Cumings made the link with copper accumulation in both the liver and the brain in 1948.[3] The first effective oral chelation agent, penicillamine, was discovered in 1956 by British neurologist John Walshe.[4]

Genetics

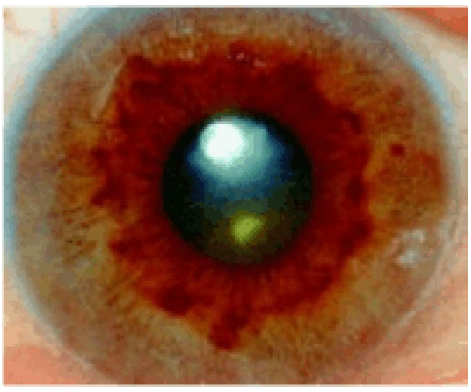
The Wilson's disease gene (*ATP7B*) has been mapped to chromosome 13 (13q14.3) and is expressed primarily in the liver, kidney, and placenta. The gene codes for a P-type.[5] The condition is inherited in an autosomal recessive pattern. In order to inherit it, both of the parents of an individual must carry an affected gene. Most have no family history of the condition. People with only one abnormal gene are called carriers (heterozygotes) and



may have mild, but medically insignificant, abnormalities of copper metabolism.[6]

Pathophysiology

Copper is needed by the body as a cofactor for a number of enzymes such as ceruloplasmin, cytochrom c oxidase, dopamine â-hydroxylase, superoxide dismutase and tyrosinase. Copper enters the body through the digestive tract. A transporter protein on the cells of the small bowel, copper membrane transporter 1 (CMT1), carries copper inside the cells, where some is bound to metallothionein and part is carried by ATOX1 to an organelle known as the trans-Golgi network. Here, in response to rising concentrations of copper, an enzyme called ATP7A releases copper into the portal vein to the liver. Copper accumulates in the liver tissue; ceruloplasmin is still secreted, but in a form that lacks copper (termed apoceruloplasmin) and rapidly degraded in the bloodstream. When the amount of copper in the liver overwhelms the proteins that normally bind it, it causes oxidative damage through a process known as Fenton chemistry; this damage eventually leads to chronic active hepatitis, fibrosis (deposition of connective tissue) and cirrhosis. The liver also releases copper into the bloodstream that is not bound to ceruloplasmin. This free copper precipitates throughout the body but particularly in the kidneys, eyes and brain. In the brain, most copper is deposited in the basal ganglia, particularly in the putamen and globus pallidus.[7]



Clinical Features

Tiredness, portal hypertension, enlargement of the spleen, ascites, behavioral changes, depression, anxiety and psychosis.[2] About 5% of all people are diagnosed only when they develop fulminant acute liver failure, often in the context of a hemolytic anaemia. This leads to abnormalities in protein production and metabolism by the liver. The deranged protein metabolism leads to the accumulation of waste products such as ammonia in the bloodstream. When these irritate the brain, the person develops hepatic encephalopathy.[2]

Other Organs Involvement

1. Eyes: *Kayser–Fleischer Rings* (KF rings) around the iris due to copper deposition in Descemet's membrane of the cornea, visible by slit lamp examination.[2]
2. Kidneys: renal tubular acidosis leads to nephrocalcinosis.[2]
3. Heart: cardiomyopathy is a rare.[2]

Diagnosis

Liver function tests: Raised aspartate transaminase, alanine transaminase and bilirubin level, the prothrombin time may be prolonged, Alkaline phosphatase levels are relatively low in those with Wilson's-related acute liver failure.[2]

Ceruloplasmin: Levels of ceruloplasmin are abnormally low (<0.2 g/L) in 80–95% of cases.[6] The combination of neurological symptoms, Kayser–Fleisher rings and a low ceruloplasmin level is considered sufficient for the diagnosis of Wilson's disease.[6]

Serum and Urine Copper: Serum copper is paradoxically low but urine copper is elevated in Wilson's disease. Urine is collected for 24 hours in a bottle with a copper-free liner. Levels above 100 ìg/24 h (1.6 ìmol/24 h) confirm Wilson's disease, and levels above 40 ìg/24 h (0.6 ìmol/24 h) are strongly indicative).[6]

Liver Biopsy: The gold standard or most

ideal test is a liver biopsy.[2]

Magnetic Resonance Imaging (MRI) of the brain

If there are neurological symptoms is usually performed; this show shy perintensities in the part of the brain called the basal ganglia in the T2 setting. MRI may also demonstrate the characteristic "face of the giant panda" pattern.[6]

Treatment

Dietary

A diet low in copper-containing foods is recommended with the avoidance of mushrooms, nuts, chocolate, dried fruit, liver, and shellfish.[2]

Medication

Generally, penicillamine is the first treatment used. This binds copper (chelation) and leads to excretion of copper in the urine.[2] Intolerant to penicillamine may instead be commenced on trientine hydrochloride or tetrathiomolybdate. Once all results have returned to normal, zinc may be used instead of chelators to maintain stable copper levels in the body. Zinc stimulates metallothionein, a protein in gut cells that binds copper and prevents their absorption and transport to the liver.[6]

Physiotherapy can assist in coping with ataxia, dystonia and tremors, as well as preventing the development of contractures that can result from dystonia.

Liver transplantation is an effective cure for Wilson's disease, but is used only in particular scenarios because of the numerous risks and complications associated with the procedure.[6]

Conclusion

Family members of patient with proven cases requires screening for presymptomatic Wilson's disease. Such screening should include determination of the serum Ceruloplasmin level and urinary Copper excretion. In asymptomatic siblings of affected patient early institution of chelation or zinc therapy can prevent expression of disease.

References

1. William F Balistren and Rebeca G. Careyet el. Nelsson textbook of paediatrics 19th edition. 1391-92.
2. Ala A, Walker AP, Ashkan K, Dooley JS, Schilsky ML. Wilson's disease. *Lancet.* 2007; 369(9559): 397–408.
3. Walshe JM. Treatment of Wilson's disease: the historical background. *QJM.* 1996; 89(7): 553–5.
4. Brewer GJ, Askari FK. Wilson's disease: clinical management and therapy. *Journal of Hepatology.* 2005; 42(Suppl 1): 13–21. doi :10.1016/j.jhep. 2004.11.013.
5. Merle U, Schaefer M, Ferenci P, Stremmel W. Clinical presentation, diagnosis and long term outcome of Wilson's disease: a cohort study. *Gut.* 2007; 56(1): 115–20. doi :10.1136/gut. 2005.087262.
6. Roberts EA, Schilsky ML. A practice guideline on Wilson disease (PDF). *Hepatology.* 2003; 37(6): 1475.
7. de Bie P, Muller P, Wijmenga C, Klomp LW. Molecular pathogenesis of Wilson and Menkes disease: correlation of mutations with molecular defects and disease phenotypes. *J Med Genet.* 2007; 44(11): 673–88. doi :10.1136/jmg.2007.052746.

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Brachio-Otic Syndrome

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Abstract

Branchio-Otic syndrome is a rare autosomal dominant disorder characterized by syndromic association of branchial cysts or fistulae along with external, middle & inner ear malformations.

Keywords: Brachio-Otic Syndrome; BOS1; BOS2; BOS3; EYA1; Hearing loss; SIX1.

Introduction

Branchio-otic syndrome (BOS) is an autosomal dominant disorder clinically diagnosed by (1) at least three major criteria including branchial anomalies, deafness, preauricular pits, (2) two major criteria, and at least two minor criteria including external ear anomalies, middle ear anomalies, inner ear anomalies, preauricular tags, facial asymmetry, and palate abnormalities, or (3) one major criterion and an affected first-degree relative who meets the criteria for BOS syndrome. A similar syndrome associated with renal anomalies is called as Brachio-Otic-Renal syndrome.

Case Summary

An eight year old girl was admitted in our hospital with complaints of multiple swellings in both pre-auricular regions since birth and hearing loss since last 6 months. The swellings were small in size as like wheat grains initially, which then gradually increased over period to present size of almonds. The swellings were painless and without any signs of inflammation. Parents also noticed her hearing loss about 6 months back which was gradually progressing. Parents gave history of repeated attacks of upper respiratory tract infection. There was no any history of earache, ear discharge, fever, etc. Also parents didn't give any history of trauma or operative procedure over external ears. She was operated for cleft lip four years back. Her parents had family history of consanguineous marriage. She was a full term normal hospital delivery with good cry and adequate weight at birth. Our case was first issue of parents out of their four siblings (3 females and a male); none of them having any congenital anomaly.

On examination she was an average built girl with mild pallor. All vital parameters were within normal limits as per her age. On head to toe examination, she was having a pre-auricular pit on left side. Also she was having bilateral multiple pre-auricular tags. On left side, there were three pre-auricular tags of sizes 1.5*0.75 cm, 1*0.5 cm and 0.5*0.5 cm on right side; a single pre-auricular tag of size 1.5*0.75 cm was present. No any other externally detectable anomaly was present over body. Systemic examination also was

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Table: Clinical features

Clinical features	BOR syndrome	BOS	Our patient
Branchial cleft fistulas	+	+	-
Renal anomalies	+	-	-
Lacrimal duct stenosis	+	+	-
Hearing impairment	+	+	+
Ear defect	+	+	-
Preauricular pits	+	+	+
Preauricular tag	+	+	+
Facial asymmetry	+	+	+
Development delay	+	+	+
Microsomia	+	+	-
Impaired speech	+	+	+
Cleft lip/palate	+	+	+
Tracheoesophageal fistula	+	+	-
Heart defect	+	+	-
Genito-urinary anomalies	+	-	-
Irregular skull shape	+	+	+
Mandibular hypoplasia	+	+	+
Micrognathia	+	+	+
Recessed jaw	+	+	+
Facial nerve paralysis	+	-	-
Central nervous system defects	+	-	-

within normal limits. Opinion from ENT department was taken which, after audiometric test, stated sensory neural hearing loss from left ear.

Following investigations were done.

Hb – 10.7 gm%; Total leukocyte count – 7500/cmm; (N-49, L-41, E-08, M-02, B-00); Platelet count – 2.34 lacs/cmm; ESR (Westergren) – 10 mm at the end of one hour; Blood urea – 19 mg%; Sr. Creatinine – 0.8 mg/dl; Sr. Sodium – 136 mmol/L; Sr. Potassium – 3.8 mmol/L.

Fig 1: Pre-auricular pit

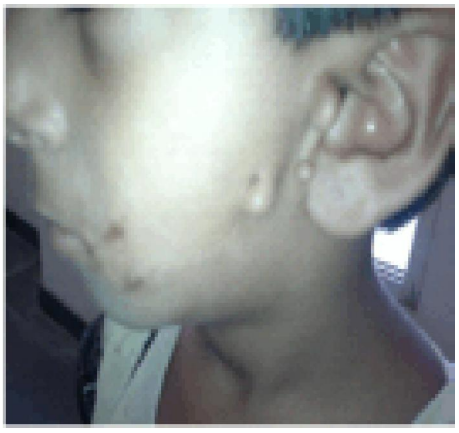
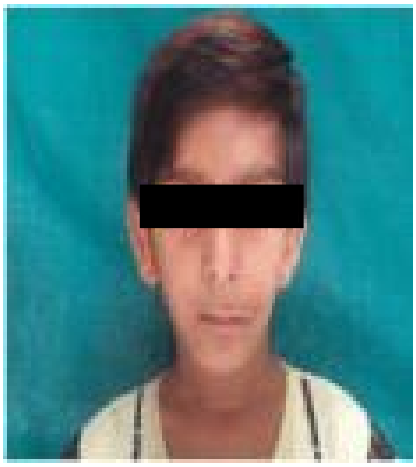


Fig 2: Facial asymmetry



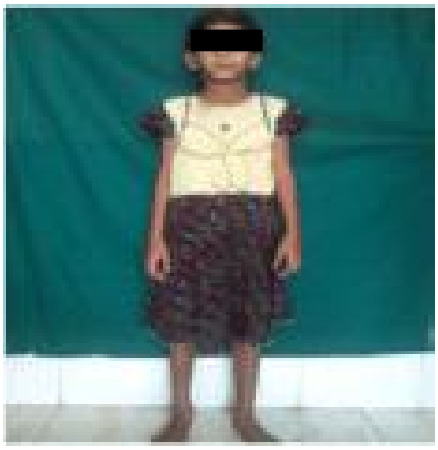
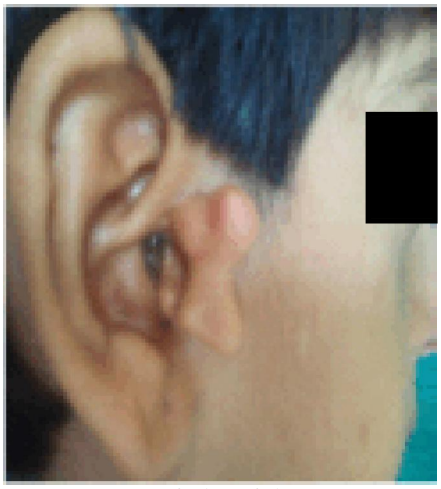
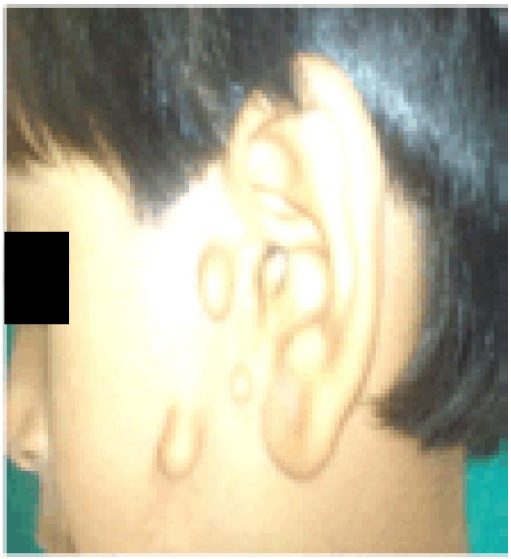


Figure 3: Pre-auricular tags

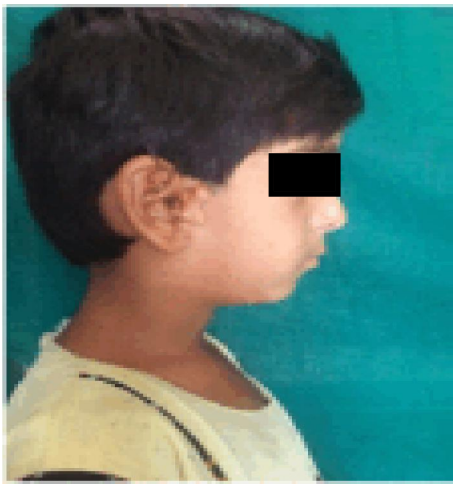


Right side

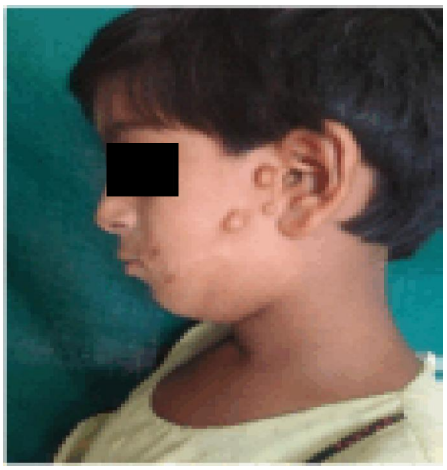


Left side

Figure 4: Recessed Jaw



Right lateral



Left lateral

Ultrasonography of abdomen and pelvis shows findings within normal limits.

X-ray chest (PA view) didn't show any abnormality.

CT Brain (Plain) was also within normal limits.

Patient was discharged and was advised follow up after 05 days as per surgery appointment.

Discussion

Branchio-otic syndrome (BOS) is a genetic condition that typically disrupts the development of tissues in the neck and causes malformations of the ears. The signs and symptoms of this condition can vary, however.

"Branchio-" refers to the second branchial arch, which is a structure in the developing embryo that gives rise to tissues in the front and side of the neck. In people with branchio-otic syndrome, abnormal development of the second branchial arch can result in the formation of masses in the neck called branchial cleft cysts. In some people, abnormal connections called fistulae form passages between these cysts and the surface of the neck. Fistulae can also develop between the skin of the neck and the throat, near the tonsils. Branchial cleft cysts and fistulae can cause medical problems if they become infected.

"Otic-" refers to the ear; most people with

branchio-otic syndrome have hearing loss and other ear abnormalities. The hearing loss is known as sensorineural deafness if it is caused by changes in the inner ear, and conductive deafness if it is caused by changes in the middle ear. Branchio-otic syndrome can also involve hearing loss that results from changes in both the inner ear and the middle ear, which is called mixed hearing loss. Other ear abnormalities associated with branchio-otic syndrome include malformations of the inner ear or middle ear and abnormally shaped outer ears (pinnae). Some affected people also have tiny holes in the skin (preauricular pits) or small flaps of skin (preauricular tags) just in front of the ear.

When the above clinical features are associated with renal anomalies is called as Brachiootorenal (BOR) syndrome. In that, "Renal" refers to the abnormalities of kidney structure and function. These abnormalities range from mild to severe and can affect one or both kidneys. In some cases, end-stage renal disease (ESRD) develops later in life. This serious condition occurs when the kidneys become unable to filter fluids and waste products from the body effectively.

Branchiootorenal syndrome (also called as Melnick-Fraser syndrome) affects about 1 in 40,000 people.

Pre-auricular tags are also found in oculo-auriculovertebral syndrome (OAVS) , also known as Goldenhar's syndrome. As ocular and vertebral components are not present in this case, so it is ruled out.

Molecular Biology

Branchio-otic syndrome (BOS) is an autosomal dominant disorder caused by mutations in the EYA1 gene, a human homolog of the Drosophila eyes absent gene (Eya). A second locus for BOS was localized to chromosome 1q31. Recently, a third gene locus for BOS to 14q21.3–q24.3 by linkage study is mapped and designated it as branchiootic syndrome 3 (BOS3). This chromosomal region contains the SIX1, SIX4, SIX6 gene cluster, the products of which are known to act in a developmental pathway of the EYA genes and OTX2. Mutations in SIX1 have been reported in patients with BOS, thus identifying SIX1 as a gene causing BOS.

References

1. Schwartz CE, Smith RJ. Branchio-oto-renal syndrome: The mutation spectrum in EYA1 and its phenotypic consequences. *Hum Mutat*. 2004; 23:582–589.
2. Fraser FC, Ling D, Clogg D, Nogrady B. Genetic aspects of the BOR syndrome—Branchial fistulas, ear pits, hearing loss,and renal anomalies. *Am J Med Genet*. 1978; 2: 241–252.
3. Hone SW, Smith RJ. Genetics of hearing impairment. *Semin Neonatol*. 2001; 6: 531–541.
4. Melnick M, Bixler D, Silk K, Yune H, Nance WE. Autosomal dominant branchio-oto-renal dysplasia. *Birth Defects Orig Artic Ser*. 1975; 11: 121–128.
5. Ruf RG, Xu PX, Silvius D, Otto EA, Beekmann F, Muerb UT, Kumar S, Neuhaus TJ, Kemper MJ, Raymond RM Jr, Brophy PD, Berkman J, GattasM, Hyland V, Ruf EM, Schwartz C, Chang EH, Smith RJ, Stratakis CA, WeilD, Petit C, Hildebrandt F. SIX1 mutations cause branchio-oto-renal syndrome by disruption of EYA1-SIX1-DNA complexes. *ProcNatlAcad Sci.* 2004; 101: 8090–8095.
6. Sensi A, Cocchi G, Martini A, Garani G, Trevisi P, Calzolari E. Branchio-oto (BO) syndrome and oculo-auriculo-vertebral phenotype: Overlapping clinical findings in a child from a BO family. *Clin Genet.* 1996; 49:300–302.
7. Verloes A, Seret N, Bernier V, Gonzales M, Herens C, Koulischer L. Branchial arch anomalies in trisomy 18. *Ann Genet.* 1991; 34: 22–24.
8. Vincent C, Kalatzis V, Abdelhak S, Chaib H, Compain S, Helias J, Vaneecloo FM, Petit C. BOR and BO syndromes are allelic defects of EYA1. *Eur J Hum Genet.* 1997; 5:242–246.

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Genetic Factors in Diabetic Peripheral Neuropathy: From the Known to the Unknown

Kumar Senthil P., MPT*; Adhikari Prabha, MD; Jeganathan, P.S., PhD*****

Abstract

The aim of this letter is directed to exploring the underlying evidence for the role of genetic factors in development of a common microvascular complication of diabetes mellitus (DM), the diabetic peripheral neuropathy (DPN). Genetic factors played a pathogenetic, predisposing role and also a protective therapeutic role in development and amelioration of DPN respectively.

Keywords: Genetic endocrinology; Molecular metabolism; Diabetic neuropathy; Neurobiochemical markers.

Dear Sir,

This letter to editor brings to you and the Indian Journal of Genetics and Molecular Research the warmest best wishes in its maiden effort to provide updated scientific information in the field of Molecular Biology from a wider inter-disciplinary evidence-informed perspective. The aim of this letter is directed to exploring the underlying evidence for the role of genetic factors in development of a common microvascular complication of diabetes mellitus (DM), the diabetic peripheral neuropathy (DPN).

Historically, experimentally induced neuropathy models had utilized genetic polymorphisms and had studied the efficacy of treatments such as Ganglioside (Gorio *et al*, 1984).[1] Initial case-control studies could not find association of genetic factors with DPN and Boulton *et al* (1984)[2] who studied 41 subjects with DPN and 41 DM subjects without PN and compared the acelyator status via HLA-A, B, C and DR antigens, could not find any significant difference between the two groups in the proportion of fast and slow acetylators. The distribution of HLA frequencies was also similar in subjects with and without neuropathy for both Type 1 (insulin-dependent) and Type 2 (non-insulin-dependent) diabetic patients.

17 years later, Benjafield *et al* (2001)[3] identified genetic variation in the tumor necrosis factor (TNF) receptor 2 gene (TNFRSF1B)with polymorphism of CA16 allele which was also previously associated with insulin resistance in type 2 diabetes, hypercholesterolemia, coronary artery disease, and essential hypertension. Another study by Strokov*et al* (2003)[4] examined polymorphic markers Ala(-9)Val in SOD2 gene and Arg213Gly in SOD3 gene and their relationship to DPN and found that genes encoding the enzymes Mn-SOD and extracellular superoxide dismutase (EC-SOD) were associated with the pathogenesis of DPN.

More recently, Gazzaruso *et al* (2012)[5]

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studied association of Lipoprotein(a)-Lp(a)- and homocysteine (Hcy) with diabetic foot ulcerations, which were classified according to the presence of peripheral artery disease (PAD) or neuropathy. The study found that high Lp(a) and Hcy levels were associated with the development of vascular diabetic foot (VDF), while low Lp(a) levels appear to be associated with delayed wound healing in patients with neuropathic foot ulcerations.

Genetic factors also played a protective role and Angiotensin-converting enzyme gene single polymorphism was shown to act as a protective genetic biomarker of DPN as demonstrated by Jurado *et al* (2012)[6] who analyzed angiotensin-converting enzyme (ACE) gene polymorphism (D/I) as a genetic marker of risk of developing DPN, and found presence of ACE polymorphism heterozygous genotype D/I in 60.77% which was also independently associated with a decreased risk of DPN. Another study by Walwyn *et al* (2006)[7] investigated the effect of localized nerve growth factor (NGF) expression in a genetic mouse model of progressive diabetic neuropathy, and found that site-specific delivery of NGF initially delayed the appearance of hypoalgesia, which suggested that NGF-based gene therapy would be viable therapeutic option.

Thus genetic factors played a pathogenetic, predisposing role and also a protective therapeutic role in development and amelioration of DPN respectively. Can future controlled clinical trials explore the molecular mechanisms behind use of commonly recommended interventions for DPN?

References

1. Gorio A, Aporti F, Di Gregorio F, Schiavinato A, Siliprandi R, Vitadello M. Ganglioside treatment of genetic and alloxan-induced diabetic neuropathy. *Adv Exp Med Biol*. 1984; 174: 549-64.
2. Boulton AJ, Worth RC, Drury J, HardistyCA, Wolf E, Cudworth AG, *et al*. Genetic and metabolic studies in diabetic neuropathy. *Diabetologia*. 1984; 26(1): 15-9.
3. Benjafield AV, Glenn CL, Wang XL, Colagiuri S, Morris BJ. TNFRSF1B in genetic predisposition to clinical neuropathy and effect on HDL cholesterol and glycosylated hemoglobin in type 2 diabetes. *Diabetes Care*. 2001; 24(4): 753-7.
4. Strokov IA, Bursa TR, Drepa OI, Zotova EV, Nosikov VV, Ametov AS. Predisposing genetic factors for diabetic polyneuropathy in patients with type 1 diabetes: a population-based case-control study. *ActaDiabetol*. 2003; 40(Suppl 2): S375-9.
5. Gazzaruso C, Coppola A, Montalcini T, Baffero E, Garzaniti A, Pelissero G, *et al*. Lipoprotein(a) and homocysteine as genetic risk factors for vascular and neuropathic diabetic foot in type 2 diabetes mellitus. *Endocrine*. 2012; 41(1): 89-95.
6. Jurado J, Ybarra J, Romeo JH, Garcia M, Zabaleta-Del-Olmo E. Angiotensin-converting enzyme gene single polymorphism as a genetic biomarker of diabetic peripheral neuropathy: longitudinal prospective study. *J Diabetes Complications*. 2012; 26(2): 77-82.
7. Walwyn WM, Matsuka Y, Arai D, Bloom DC, Lam H, Tran C, *et al*. HSV-1-mediated NGF delivery delays nociceptive deficits in a genetic model of diabetic neuropathy. *Exp Neurol*. 2006; 198(1): 260-70.

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The Role of Complement Factors in Neurodegeneration, Neuroinflammation and Neuroprotection: Friend or Foe?

Kumar Senthil P., MPT*; Adhikari Prabha, MD; Jeganathan, P.S., PhD*****

Abstract

Complement is a major component of innate immune system involved in defending against all the foreign pathogens through complement fragments that participate in opsonization, chemotaxis, and activation of leukocytes and through cytolysis by C5b-9 membrane attack complex. The complement (C) system has been implicated as a factor in the causation or propagation of tissue injury in central and peripheral nervous system disorders. Complement factors play a predominant role in nerve injury, inflammation and repair, thus playing part in neurodegeneration, neuroinflammation and neuroprotection.

Keywords: Immunology; Neuroimmunology; Immunogenetics; Genetic neurology; Complement system.

Complement is a major component of innate immune system involved in defending against all the foreign pathogens through complement fragments that participate in opsonization, chemotaxis, and activation of leukocytes and through cytolysis by C5b-9 membrane attack complex.[1] The complement (C) system has been implicated as a factor in the causation or propagation of tissue injury in central and peripheral nervous system disorders.[2]

Complement opsonins (C1q, C3b, and iC3b) interact with surface complement receptors to promote phagocytosis, whereas complement anaphylatoxins C3a and C5a initiate local inflammatory responses by taking part in humoral and cellular immunity mechanisms of neurodegeneration and neuroprotection involved in cytolysis and immune/ inflammatory responses.[3]

Many studies had reported their role in central nervous system disorders like encephalomyelitis, multiple sclerosis, Alzheimer's disease.[4,5] Rus and Nicolescu[6] described the role of complement factors as; "Myelin and oligodendrocyte (OLG) activate the classical pathway of complement in vitro in the absence of antibodies. Sublytic C5b-9 in the absence of cell death induces proto-oncogenes, activates cell cycle, and enhances cell survival in OLG. In addition, C5b-9 reverses the differentiation phenotype in OLG and enhances cell survival. Beta amyloid protein is an activator of the complement system and neurons are susceptible to bystander complement mediated damage."

Astrocytes, ependymal cells, endothelial cells, microglia, and neurons synthesize various complement proteins or express complement receptors on their cell surfaces, and binding of proteolytic fragments derived from activation of complement by specific receptors leads to responses towards inflammation, opsonization, and B-cell activation.[7]A fine balance of C activation and regulation mediated the elimination of invading pathogens and the protection of the host from excessive C deposition on healthy tissues, and upon disruption of this delicate balance, the C system may cause injury and

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contribute to the pathogenesis of various diseases, including peripheral neuropathies.[8]

Complement cascade factors of classical (C1qa, C1qb, C1qc, C2 and C4) and alternative (C3, B and adipsin) pathways were known to play a critical role in myelin clearance after peripheral nerve injury.[9] The receptors located within the nerve fascicles are probably of glycoprotein nature and the receptors for C3b in peripheral nerve tissues may be of significance in the deposition of immune complexes, thus playing a role in acute polyradiculoneuritis.[10]

Furthermore, activation of complement by peripheral nerve myelin (PNM) *via* the alternative pathway was shown by cleavage of C3 in normal human serum (NHS) and of B in C2-deficient serum (C2d-HS). Increasing consumption of hemolytic activity of C3 in Mg-EGTA-treated NHS was also noted with increasing amounts of PNM as a consequence of a variety of pathologic conditions affecting the peripheral nervous system.[11]

RNA (RT-PCR and northern blot hybridization) and protein (western blot analysis and immunohistochemistry) studies confirmed high expression of classical pathway components, alternative pathway components and inhibitory components in sciatic nerve (first components of complement in axons, inhibitory components in perineurium) to protect the nerve from a complement attack.[12]

Complement factors play a predominant role in nerve injury, inflammation and repair.[13] Expression of complement and clusterin were prominent features of neural degeneration and regeneration and they provide useful insights into potentially new therapeutic approaches in neurodegenerative disorders.[14] Most of the studies were on experimental rodent models, and future clinical trials are needed to establish a bedside evidence to relate it into clinical practice.

References

1. Rus H, Cudrici C, Niculescu F. The role of the complement system in innate immunity.*Immunol Res*. 2005; 33(2): 103-12.
2. Morgan BP, Gasque P, Singhrao S, Piddlesden SJ. The role of complement in disorders of the nervous system. *Immunopharmacology*. 1997; 38(1-2): 43-50.
3. van Beek J, Elward K, Gasque P. Activation of complement in the central nervous system: roles in neurodegeneration and neuroprotection. *Ann N Y Acad Sci*. 2003; 992: 56-71.
4. Barnum SR. Complement in central nervous system inflammation. *Immunol Res*. 2002; 26(1-3): 7-13.
5. Rus H, Cudrici C, David S, Niculescu F. The complement system in central nervous system diseases. *Autoimmunity*. 2006; 39(5): 395-402.
6. Rus H, Niculescu F. The complement system in central nervous system diseases. *Immunol Res*. 2001; 24(1): 79-86.
7. Barnum SR. Complement biosynthesis in the central nervous system. *Crit Rev Oral Biol Med*. 1995; 6(2): 132-46.
8. Ramaglia V, Daha MR, Baas F. The complement system in the peripheral nerve: friend or foe? *MolImmunol*. 2008; 45(15): 3865-77.
9. Chrast R, Verheijen MH, Lemke G. Complement factors in adult peripheral nerve: a potential role in energy metabolism. *Neurochem Int*. 2004; 45(2-3): 353-9.
10. Nyland H, Matre R, Tönder O. Complement receptors in human peripheral nerve tissue. *Acta Pathol Microbiol Scand C*. 1979; 87C(1): 7-10.
11. Koski CL, Vanguri P, Shin ML. Activation of the alternative pathway of complement by human peripheral nerve myelin. *J Immunol*. 1985; 134(3): 1810-4.
12. de Jonge RR, van Schaik IN, Vreijling JP, Troost D, Baas F. Expression of complement components in the peripheral nervous system. *Hum Mol Genet*. 2004; 13(3): 295-302.
13. Morgan BP, Gasque P, Singhrao SK, Piddlesden SJ. Role of complement in inflammation and injury in the nervous system. *Exp Clin Immunogenet*. 1997b; 14(1): 19-23.
14. Törnqvist E, Liu L, Aldskogius H, Holst HV, Svensson M. Complement and clusterin in the injured nervous system. Neurobiol Aging. 1996; 17(5): 695-705.

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[2] Twetman S, Axelsson S, Dahlgren H, Holm AK, Källestål C, Lagerlöf F, et al. Caries-preventive effect of fluoride toothpaste: A systematic review. Acta Odontol Scand 2003;61:347-55.

Article in supplement or special issue

[3] Fleischer W, Reimer K. Povidone iodine antisepsis. State of the art. Dermatology 1997;195 Suppl 2:3-9.

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[4] American Academy of Periodontology. Sonic and ultrasonic scalers in periodontics. J Periodontol 2000;71:1792-801.

Unpublished article

[5] Garoushi S, Lassila LV, Tezvergil A, Vallittu PK. Static and fatigue compression test for particulate filler composite resin with fiber-reinforced composite substructure. Dent Mater 2006.

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[6] Hosmer D, Lemeshow S. Applied logistic regression, 2nd edn. New York: Wiley-Interscience; 2000.

Chapter in book

[7] Nauntofte B, Tenovuo J, Lagerlöf F. Secretion and composition of saliva. In: Fejerskov O, Kidd EAM, editors. Dental caries: The disease and its clinical management. Oxford: Blackwell Munksgaard; 2003. p. 7-27.

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[8] World Health Organization. Oral health surveys - basic methods, 4th edn. Geneva: World Health Organization; 1997.

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[9] National Statistics Online—Trends in suicide by method in England and Wales, 1979-2001. www.statistics.gov.uk/downloads/theme_health/HSQ 20.pdf (accessed Jan 24, 2005): 7-18. Only verified references against the original documents should be cited. Authors are responsible for the accuracy and completeness of their references and for correct text citation. The number of reference should be kept limited to 20 in case of major communications and 10 for short communications.

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