Cent. Eur. J. Med. • 6(4) • 2011 • 435-441
DOI: 10.2478/s11536-011-0039-8

VERSITA

Central European Journal of **Medicine**

A Population-based Case Control Study of Congenital Abnormalities and Medication Use During Pregnancy Using the Czech National Register of Congenital Abnormalities

Research Article

Jana Mašková[1], Antonín Šípek[2], Peter Kollár[1,*]

[1] *Department of Human Pharmacology and Toxicology, Faculty of Pharmacy, University of Veterinary and Pharmaceutical Sciences Brno, Palackého 1-3, CZ-612 42, Brno, Czech Republic*

[2] *Department of Medical Genetics, Thomayer University Hospital, Vídeňská 800, CZ-140 59, Prague, Czech Republic*

Received 25 August 2010; Accepted 26 April 2011

Abstract: The aim was to identify and quantify the association between the use of particular medications during the first trimester of pregnancy and selected congenital abnormalities (CAs) of newborns. Data were from the Czech National Registry of CAs. We used a case-control design , and collected total of 7285 cases and 9143 controls. Thiethylperazine and iron compounds had no effect on development of CAs. Lower odds ratio and potentially protective associations were found between CAs and bioflavonoids, folic acid, progesterone, levothyroxine, and iodine therapy. Since the protective effect of bioflavonoids was not described before, analysis of interaction with other drugs was performed. However, their protective effect was not confirmed and the strongest significant protective effect was detected in combination of bioflavonoids and progesterone. Increased odds ratio were identified for hydroxyprogesterone, phenoxymethylpenicillin, aspirin, paracetamol and valproic acid. The association between paracetamol and congenital foot deformities was not significant, while the same association for the whole group of CAs and deformities of musculoskeletal system had significantly increased odds ratio. Except newly described effect of bioflavonoids, our results are in agreement with risk categories defined by health authorities in USA and Australia, and with results of other studies. According to our results, paracetamol does not influence development of congenital foot deformities.

Keywords: *Bioflavonoids • Congenital abnormalities • Drug use • Pregnancy • Teratology*

1. Introduction

Congenital anomalies are a serious medical and social problem. About 10 % of congenital defects are caused by exogenous factors having effects on the developing embryo [1], presenting a potential opportunity to prevent a range of congenital anomalies [2]. For 50-60% of congenital defects, on the other hand, the causal agent is not known. In these cases, they are caused by a combination of factors [1]. The teratogenic effect of various agents depends not only on their physical and chemical characteristics, but is also influenced by the dose, means of administration and gestational timing of exposure. The occurrences of other, concurrent exposures as well as the biological susceptibility of the mother and embryo or fetus also represent factors that may determine whether or not a particular exposure is likely to produce damage in a particular instance. The risk of major congenital anomalies in the general population is about 3-5%, but it may be much greater if the woman is at increased risk because of her age, positive family history, medical condition, or other exposures such as smoking, alcohol etc. [2]. As it is not ethically possible to test the teratogen risk on the developing fetus and as it is difficult to extrapolate findings in studies with laboratory animals, (although they help to explain

* *E-mail: kollarp@vfu.cz*

basic mechanisms of embryonic development and teratogen effect), epidemiological studies are the most important source of information. To assess risk of using specific drug during pregnancy, the Food and Drug Administration (FDA) in USA and the Australian Drug Evaluation Committee in Australia defined different risk categories of drugs according to their safety for prenatal development. Briggs et al. [3] summarized many papers concerning the risk of most frequently used drugs. There are also few other comprehensive reviews describing action of teratogenic agents [4-6]. To our knowledge, we are the first to have evaluated the effect of bioflavonoids (ATC group C05CA51) on development of the human embryo. In the Czech Republic, this group of drugs is often used to stop vaginal bleeding at the very beginning of pregnancy.

The aim of this study was to study influence of drug administration during the first trimester of pregnancy on the outcome of the newborn. We undertook a case-control study to identify and quantify associations between the use of particular drugs and selected congenital abnormalities (CAs) of newborns.

2. Material and Methods

The Czech Congenital Abnormality Registry is a national-wide registry. Physicians are required to record any observed CA as well as completing a questionnaire with the mother of the child concerning exposure to any drugs during the first trimester of pregnancy. The maximum number of CAs for each child is limited to four, and the maximum number of drugs is three. The dataset for this study, included entries in the Czech Congenital Abnormality Registry between years 1996 and 2003. We decided to consider each reported CA, rather than each child, as a case. If the mother of the child with CA was exposed to three different drugs, the CA was considered separately for each drug. CAs were diagnosed according to International Classification of Diseases. Our study population was 7285 cases of CAs. We selected to study CAs of urinary system and CAs and deformities of musculoskeletal system, because they are numerous and the critical period of these CAs is within the first trimester of pregnancy. For the larger group of CAs, which was CAs and deformities of musculoskeletal system, we could also determine associations for subgroups congenital foot deformities, polydactyly and syndactyly. Selected CAs are summarized in Table 1. Drugs were sorted according to anatomical-therapeutical-chemical (ATC) groups. Since Ascorutin® (rutosidum trihydricum 20 mg, acidum ascorbicum 100 mg) is the most prescribed drug in the group of bioflavonoids (total of 18 prescribed bioflavonoids in case group – 12 Ascorutin®, 6 Anavenol® and total of 75 prescribed bioflavonoids in control group – 72 Ascorutin®, 1 Anavenol®, 2 Venoruton®), we counted associations for Ascorutin® separately.

Table 1. Selected Congenital Abnormalities (CAs), their code according to International Classification of Diseases and numbers of cases exposed to any drug and total number of cases.

Diagnosis	code	exposed	total
CAs of urinary system	Q600-649	126	2190
CAs and deformities of musculoskeletal system	Q660-760	290	5095
Congenital foot deformities	Q660-669	102	2589
Polydactyly	Q690-699	63	999
Syndactyly	Q700-709	53	828

As controls, children without any defect born in the Institute for the Care of Mother and Child in Prague between 2001 and 2003 were considered. Information about exposure to any drugs during the first trimester of pregnancy was collected from the mothers during their stay in this hospital. We considered a maximum of three drugs per a child. Our study population consisted of 9 143 controls, 496 of which were exposed to any drug. The association between drug exposure and the occurrence of each CA was evaluated by odds ratio (OR). Using Woolf's technique, 95% confidence intervals (CI) were determined. The χ^2 test was counted to evaluate changes in frequency of prescribed drugs during the time (see Discussion). We determined associations only when the sum of cases and controls was higher than 10 and when each of them was higher than three to avoid any mistakes caused by small numbers.

3. Results

We evaluated associations between drug exposure and occurrence of CAs and we sorted results by CA (Table 2) and by drug (Table 3).

As the minimum number of controls and cases together was 10, the number of associations correlates with the number of cases in each group of CAs. The higher the number of cases, the higher the number of countable associations and consequently the higher the number of significant associations. The number of controls was the same for all groups of CAs.

The results of drug groups were influenced by the changing frequency of prescription during the years 1996-2003 (see Discussion). Despite this fact, bioflavonoids and Ascorutin® reached statistical significance in all 3 associations studied.

Table 2. Associations between drug exposure and occurrence of Congenital Abnormalities (CAs). Results sorted by diagnosis. Values with * are statistically significant at level of $P < 0.05$.

Diagnosis	ATC group	ATC code	exposed cases	exposed controls	OR	CI	
CAs of urinary system	other combinations of iron	B03AE10	3	15	0,8	0,2	2,9
	folic acid	B03BB01	4	32	0,5	0,2	1,5
	bioflavonoids	C05CA51	4	75	0,2*	0,1	0,6
	Ascorutin®	C05CA51	3	72	0,2*	0,1	0,6
	progesterone	G03DA04	4	92	0,2*	0,1	0,5
	paracetamol	N02BE01	5	16	1,3	0,5	3,6
CAs and deformities of musculoskeletal system	iron fumarate	B03AA02	5	5	1,8	0,5	6,2
	other combinations of iron	B03AE10	8	15	1,0	0,4	2,3
	folic acid	B03BB01	6	32	0,3*	0,1	0,8
	bioflavonoids	C05CA51	14	75	0,3*	0,2	0,6
	Ascorutin®	C05CA51	9	72	0,2*	0,1	0,5
	hydroxyprogesterone	G03DA03	9	4	4,0*	1,2	13,2
	progesterone	G03DA04	8	92	0,2*	0,1	0,3
	levothyroxin	H03AA01	10	53	0,3*	0,2	0,7
	iodine therapy	H03CA	4	12	0,6	0,2	1,9
	phenoxymethylpenicillin	J01CE02	14	3	8,4*	2,4	29,2
	aspirin	N02BA01	7	3	4,2*	1,1	16,2
	paracetamol	N02BE01	21	16	2,4*	1,2	4,5
	valproic acid	N03AG01	8	4	3,6*	1,1	12,0
	thiethylperazine	R06AD03	5	10	0,9	0,3	2,6
Congenital foot deformities	other combinations of iron	B03AE10	6	15	1,4	0,5	3,6
	bioflavonoids	C05CA51	8	75	0,4*	0,2	0,8
	Ascorutin®	C05CA51	4	72	0,2*	0,1	0,5
	levothyroxin	H03AA01	4	53	0,3*	0,1	0,7
	phenoxymethylpenicillin	J01CE02	7	3	8,1*	2,1	31,3
	paracetamol	N02BE01	6	16	1,3	0,5	3,3
	thiethylperazine	R06AD03	4	10	1,4	0,4	4,4
Polydactyly	folic acid	B03BB01	4	32	1,2	0,4	3,3
	levothyroxin	H03AA01	3	53	0,5	0,2	1,7
	paracetamol	N02BE01	5	16	2,9*	1,1	7,9
Syndactyly	paracetamol	N02BE01	7	16	4,9*	2,0	11,9

Neither thietylperazine nor iron compounds had any relationship to the development of CAs. Protective associations were observed between CAs and folic acid (1 significant association of 3), progesterone (2 of 2), levothyroxine (3 of 3) and iodine therapy. Increased OR were found for hydroxyprogesterone (1 significant association of 1), phenoxymethylpenicillin (2 of 2), aspirin (1 of 1), paracetamol (3 of 5) and valproic acid (1 of 1).

We performed detailed analysis of bioflavonoids interaction with the other drugs to find out more about their potential protective effect. For this analysis we pooled both groups of CAs those of the urinary system and deformities of musculoskeletal system to avoid any mistake caused by small numbers. Out of 75 controls exposed to bioflavonoids, 21 were exposed to bioflavonoid only, 36 to bioflavonoid and progesterone and 18 to bioflavonoid and other drugs. Out of 15 cases exposed to bioflavonoids, 8 were exposed to bioflavonoid only, 2 to bioflavonoid and progesterone and 8 to bioflavonoid and various other drugs. We performed additional analysis of these selected groups and analysis of progesterone itself and progesterone and various other drugs (see Table 4).

Groups of bioflavonoids only and bioflavonoids and various other drugs were protective, but not statistically significant. The other examined associations were significant with protective effect.

Table 3. Associations between drug exposure and occurrence of Congenital Abnormalities (CAs). Results sorted by ATC group. Values with * are statistically significant at level of $P < 0.05$.

ATC group	diagnosis code	exposed cases	unexposed cases	exposed controls	OR	CI	
iron fumarate	CAs and deformities of musculoskeletal system	5	4805	5	1,8	0,5	6,2
other combinations of iron	CAs of urinary system	3	2064	15	0,8	0,2	2,9
	CAs and deformities of musculoskeletal system	8	4805	15	1,0	0,4	2,3
	Congenital foot deformities	6	2496	15	1,4	0,5	3,6
folic acid	CAs and deformities of musculoskeletal system	6	4805	32	0,3*	0,1	0,8
	CAs of urinary system	4	2064	32	0,5	0,2	1,5
	Polydactyly	4	936	32	1,2	0,4	3,3
Ascorutin®	CAs of urinary system	3	2064	72	0,2*	0,1	0,6
	Congenital foot deformities	4	2496	72	0,2*	0,1	0,5
	CAs and deformities of musculoskeletal system	9	4805	72	0,2*	0,1	0,5
bioflavonoids	CAs of urinary system	4	2064	75	0,2*	0,1	0,6
	CAs and deformities of musculoskeletal system	14	4805	75	0,3*	0,2	0,6
	Congenital foot deformities	8	2496	75	0,4*	0,2	0,8
hydroxyprogesterone	CAs and deformities of musculoskeletal system	9	4805	4	4,0*	1,2	13,2
progesterone	CAs and deformities of musculoskeletal system	8	4805	92	0,2*	0,1	0,3
	CAs of urinary system	4	2064	92	0,2*	0,1	0,5
levothyroxin	Congenital foot deformities	4	2496	53	0,3*	0,1	0,7
	CAs and deformities of musculoskeletal system	10	4805	53	0,3*	0,2	0,7
	Polydactyly	3	936	53	0,5	0,2	1,7
iodine therapy	CAs and deformities of musculoskeletal system	4	4805	12	0,6	0,2	1,9
phenoxymethylpenicillin	Congenital foot deformities	7	2496	3	8,1*	2,1	31,3
	CAs and deformities of musculoskeletal system	14	4805	3	8,4*	2,4	29,2
aspirin	CAs and deformities of musculoskeletal system	7	4805	3	4,2*	1,1	16,2
paracetamol	Congenital foot deformities	6	2496	16	1,3	0,5	3,3
	CAs of urinary system	5	2064	16	1,3	0,5	3,6
	CAs and deformities of musculoskeletal system	21	4805	16	2,4*	1,2	4,5
	Polydactyly	5	936	16	2,9*	1,1	7,9
	Syndactyly	7	775	16	4,9*	2,0	11,9
valproic acid	CAs and deformities of musculoskeletal system	8	4805	4	3,6*	1,1	12,0
thiethylperazine	CAs and deformities of musculoskeletal system	5	4805	10	0,9	0,3	2,6
	Congenital foot deformities	4	2496	10	1,4	0,4	4,4

In the group of cases with CAs and deformities of musculoskeletal system, we could also determine associations for three subgroups of congenital foot deformities, polydactyly and syndactyly. Congenital foot deformities comprise 2589 of all 5095 cases of CAs and deformities of the musculoskeletal system. With the exception of paracetamol, associations studied in both CAs groups were comparable. While association between paracetamol and congenital foot deformities was not significant, the same association for CAs and deformities of musculoskeletal system, polydactyly and syndactyly had significantly increased OR.

4. Discussion

As the data in our study are retrospective, they could be influenced by recall and observer bias. This means that mothers of children with CAs are likely to be quizzed more intensely, and may reply more conscientiously than mothers of controls. This could result in an overestimation of drug risk by mothers whose children have CA. However, our most important results show possible protective association of bioflavonoids and CAs and no effect of paracetamol on congenital foot deformity; this type of bias would make our results even stronger. A more important problem of this study is the

Table 4. Association analysis of drug interactions. Values with * are statistically significant at level of $P < 0.05$.

drug interaction	exposed cases	unexposed cases	exposed controls	OR	CI	
bioflavonoids only	8	6869	21	0,5	0,2	1,1
bioflavonoids and progesterone	2	6869	36	0,1*	0,0	0,3
progesterone only	8	6869	27	0,4*	0,2	0,8
bioflavonoids and various other drugs	8	6869	18	0,6	0,2	1,3
progesterone and various other drugs	2	6869	29	0,1*	0,0	0,4

Table 5. Changes in drug prescription frequency during the time. Values with * are statistically significant at level of $P < 0.05$.

ATC group	ATC code	1996-1999	2000-2003	mean	c2
iron fumarate	B03AA02	20	3	11,5	12,6*
other combinations of iron	B03AE10	23	15	19	1,7
folic acid	B03BB01	12	15	13,5	0,3
bioflavonoids	C05CA51	48	30	39	4,2*
hydroxyprogesterone	G03DA03	34	5	19,5	21,6*
progesterone	G03DA04	6	21	13,5	8,3*
levotyroxin	H03AA01	22	30	26	1,2
iodine therapy	H03CA	21	8	14,5	5,8*
phenoxymethylpenicillin	J01CE02	44	19	31,5	9,9*
aspirin	N02BA01	13	3	8	6,3*
paracetamol	N02BE01	56	45	50,5	1,2
valproic acid	N03AG01	11	27	19	6,7*
thiethylperazine	R06AD03	17	8	12,5	3,2

different time period of cases and controls. Although the information about controls were collected from 2001 – 2003 we used data from cases spanning the period of 1996 – 2003. This was methodologically necessary in order to have as many cases as possible in order to make studied associations countable. On the other hand, our results could be influenced by changing frequency of drug prescription during the two time periods. If any drug was more frequently prescribed during the years 1996-2000 then during the years 2001-2003, it could result in an overestimate of drug risk. On the other hand, if a drug was more frequently prescribed during the years 2001-2003 then during the years 1996-2000, the risk estimate would be biased towards the null. To solve this question, we stratified cases according to year of birth into two groups 1996-2000 and 2001-2003 for each ATC group. To avoid mistake caused by small numbers, we added 260 exposed cases of CA (96 - orofacial clefts, 55 - cryptorchidism and 109 – hypospadias) from years 1996-2003 into this analysis. By the χ^2 analysis, we tested the hypothesis that both subgroups were of the same size. The expected value was arithmetic mean of both groups. To a 5 % level of significance, the null hypothesis is correct when the χ^2 value is less then 3,84. Results of the χ^2 test are summarized in Table 5.

Two ATC groups progesterone and valproic acid were more frequently prescribed during the years 2001-2003. Therefore, their OR should be higher then it was counted. In contrast, iron fumarate, hydroxyprogesterone, iodine therapy, phenoxymethylpenicillin and aspirin were more frequently prescribed between 1996 and 2000, and their OR should be lower then counted. Surprisingly, bioflavonoids belong to the second group and their strong protective association should be even stronger than observed. Remaining ATC groups, other combination of iron, folic acid, levothyroxine, paracetamol and thiethylperazine, did not change frequency in time.

4.1. CAs of urinary system

Well-known drugs which interfere in the development of the kidneys are inhibitors of angiotensin converting enzyme and antagonists of angiotensin II type 1 receptor [7,8]. Undoubtedly, there are more biochemical modulators in this process than the renin-angiotensin system and angiotensin receptor. However, CAs of kidney and urinary tract very often take a familial pattern as Mendelian type of inheritance or as a complex trait resulting from a minor mutation of multiple specific genes that are involved in normal embryogenesis [9]. In our study, bioflavonoids, and especially Ascorutin®,

were found to have significant protective associations. Progesterone was also significant but it was strongly influenced by changes in prescription frequency in time as described above. In contrast, no significance was observed in other combinations of iron, folic acid and notably paracetamol.

4.2. CAs and deformities of musculoskeletal system

Iron fumarate, other combinations of iron and thiethylperazine had no significant association. Iodine therapy was not found to be significant but because it was influenced by changes in prescription frequency in time, it would probably have slightly protective effect in reality. Levothyroxine has a quite strong significant protective association. Therapy either with folic acid or bioflavonoids and Ascorutin® led to significant protective associations. In our study, results of progesterone and hydroxyprogesterone were strongly influenced by changes in prescription frequency in time. In fact, the effect of these two drugs is probably neither as harmful as it appears (hydroxyprogesterone), or as protective, (progesterone). With the exception of paracetamol, all ATC groups with significant negative associations were influenced by changes in prescription frequency in time. In common population, OR of phenoxymethylpenicillin and aspirin would be lower. On the opposite, OR of valproic acid would be higher. Limb malformation belongs to the most visible and therefore most frequently reported phenotypic effect of teratogens. The specific effects of these teratogens include effect on limb morphogenesis (thalidomide, warfarin, phenytoin, valproic acid) and the effect of vascular disruption on a limb that had formed normally (misoprostol, chorionic villus sampling and phenytoin) [10].

4.3. Congenital foot deformities

Congenital foot deformities make up more than the half of cases in CAs and deformities of musculoskeletal system (2589 of 5095 cases). Therefore, it is very interesting to compare results of these two groups of CAs. For both groups, associations of other combinations of iron and thiethylperazine were not found to be significant while bioflavonoids, Ascorutin® and levothyroxine, had significantly low OR. Phenoxymethylpenicillin had also significantly high OR in both CA groups. Surprisingly, paracetamol had no significant association with congenital foot deformities, while remaining significant in all CAs and deformities of musculoskeletal system. In conclusion, paracetamol and especially acute infectious disease or fever do not likely play as important role in developing congenital foot abnormalities as in developing other CAs of musculoskeletal system, namely reduction limb defects, polydactyly and syndactyly. The most frequent congenital foot deformity, club foot (1237 of 2589 congenital foot deformity cases in our study) could be influenced by heritability as incidence is different among races and sexes. Krogsgaard et al. [11] described that also exogenic factors are pathogenic. In their study, they found increasing incidence of club foot with higher population density. Except higher number of daily person-to-person contacts leading to higher exposure to infections, many other differences between high and low population density areas (e.g. diet, lifestyle, type of work) can theoretically represent exogenous factors influencing the risk of giving birth to child with club foot. Alderman et al. [12] support the pathogenic role of exogenic factors, when they demonstrated that the OR for club foot in boys was 2.6 in mothers who smoked, compared to non-smokers.

There were not many observed cases in both groups of CAs (999 cases of polydactyly and 828 cases of syndactyly). A significant relationship with paracetamol was present in both groups. In polydactyly, association of folic acid and levothyroxine could be determined, but they were not significant. Polydactyly and syndactyly occur very often together with other malformations or even different syndromes. Talamillo et al. [13] summarized mutations of different genes responsible for polydactyly. The work of Kazy et al. [14] describes higher incidence of polydactyly and syndactyly after maternal treatment with combination of vaginal metronidazole and miconazole during second through third month of gestation (OR=6, CI 2,4-15,2).

To our knowledge, the effect of bioflavonoids (ATC group C05CA51) and namely of Ascorutin®, in embryogenesis has not been described before. As bioflavonoids were protective in most of CAs examined, their mode of action in embryogenesis has to be very general. In clinical practice, bioflavonoids are used to increase permeability and lower fragility of capillaries and these effects could be also very positive on veins of placenta. Furthermore, Ascorutin® contains 100 mg of vitamin C, which could increase maternal resistance to various infectious agents. More than one third of controls used bioflavonoids together with progesterone reflecting that such a drug combination is mostly indicated for vaginal bleeding in the beginning of pregnancy. Surprisingly, the combination of bioflavonoid and progesterone was the most protective association in our analysis. However, association analysis of drug interaction is influenced by bias caused by changes in the prescription frequency in time as well as by

small number of exposed cases. Except for the newly described effect of bioflavonoids, our results were in agreement with risk categories defined by Food and Drug Administration in the USA [3] and by Australian Drug Evaluation Committee in Australia (www.tga.gov.au) and with other previously published studies [4-6].

Acknowledgment

This work was supported by the Veterinary and Pharmaceutical University, Czech Republic (Grant number: 232/2009/FAF). The authors declare that they have no conflicts of interest.

References

[1] Moore K.L., Persaud T.V.N., The developing human: clinically oriented embryology, 8th ed., PA: Saunders/Elsevier, Philadelphia, 2008

[2] Polifka J.E., Friedman J.M., Clinical teratology: identifying teratogenic risks in humans, Clin Genet 1999, 56, 409-420

[3] Briggs G.G., Freeman R.K., Yaffe S.J., Drugs in pregnancy and lactation: a reference guide to fetal and neonatal risk, 5th ed., Lippincott Williams & Wilkins, Philadelphia, 1998

[4] Bánhidy F., Lowry R.B., Czeizel A.E., Risk and benefit of drug use during pregnancy, Int J Med Sci., 2005, 2, 100-106

[5] Brent R.L., Environmental causes of human congenital malformations: the pediatrician''s role in dealing with these complex clinical problems caused by a multiplicity of environmental and genetic factors, Pediatrics, 2004, 113, 957-968

[6] Polifka J.E., Friedman J.M., Medical genetics: 1. Clinical teratology in the age of genomics, CMAJ, 2002, 167, 265-273

[7] Daïkha-Dahmane F., Levy-Beff E., Jugie M., Lenclen R., Foetal kidney maldevelopment in maternal use of angiotensin II type I receptor antagonists, Pediatr Nephrol, 2006, 21, 729-732

[8] Chen Y., Lasaitiene D., Friberg P., The renin -angiotensin system in kidney development, Acta Physiol Scand, 2004, 181, 529-535

[9] Pope J.C., Brock J.W., Adams M.C., Stephens F.D., Ichikawa I., How they begin and how they end: classic and new theories for the development and deterioration of congenital anomalies of the kidney and urinary tract, CAKUT, J Am Soc Nephrol, 1999, 10, 2018-2028

[10] Holmes L.B., Teratogen-induced limb defects, Am J Med Genet, 2002, 112, 297-303

[11] Krogsgaard M.R., Jensen P.K., Kjaer I., Husted H., Lorentzen J., Hvass-Christensen B., et al., Increasing incidence of club foot with higher population density: incidence and geographical variation in Denmark over a 16-year period—an epidemiological study of 936,525 births, Acta Orthop, 2006, 77, 839-846

[12] Alderman B.W., Takahashi E.R., LeMier M.K., Risk indicators for talipes equinovarus in Washington State, 1987-1989, Epidemiology, 1991, 2, 289-292

[13] Talamillo A., Bastida M.F., Fernandez-Teran M., Ros M.A., The developing limb and the control of the number of digits, Clin Genet, 2005, 67, 143-153

[14] Kazy Z., Puhó E., Czeizel A.E., The possible association between the combination of vaginal metronidazole and miconazole treatment and poly-syndactyly Population-based case-control teratologic study, Reprod Toxicol, 2005, 20, 89-94