Cannabinoid findings in children hair - what do they really tell us?

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Introduction

Hair analysis for drugs and drugs of abuse is increasingly applied in child protection cases. To determine the potential risk to a child living in a household where drugs are consumed, not only can the hair of the parents be analyzed but also the hair of the child. In the case of hair analysis for cannabinoids, the differentiation between external contamination and systemic uptake is particularly difficult since the drug is quite often handled extensively prior to consumption (e.g. when preparing a joint) and smoke causes a further risk for an external contamination. Previous studies have shown that Δ9-tetrahydrocannabinolic acid A (THCA-A) detected in hair samples originate predominately from direct transfer through contaminated fingers and/or surfaces [1] and that at least parts of Δ9-tetrahydrocannabinol (THC) orginate from side-stream smoke and/or handling of the drug material [1, 2]. As THCA-A, is not incorporated into the hair matrix through the bloodstream in relevant amounts it may act as a marker for external contamination [3]. The aim of the presented study was to evalute based on the presence of THCA-A, if the positive cannabinoid results found in children hair in a child protection project are caused by external contamination.

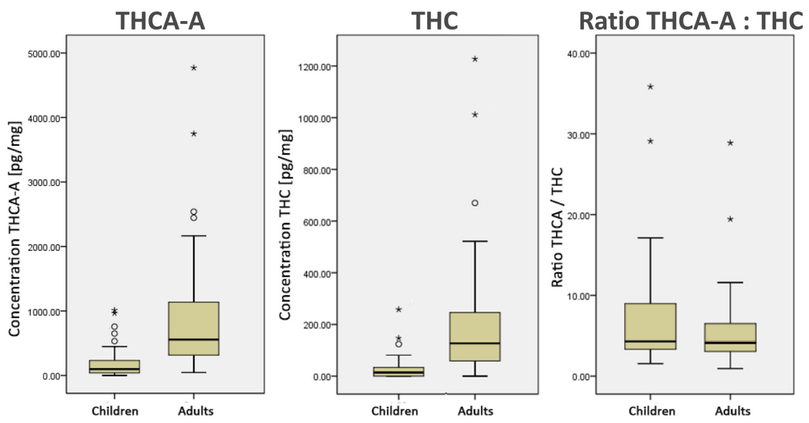
Materials and methods

Head hair samples of 41 children (age: 7 months - 12 years), 4 teenagers (age: 7 - 13 years) and 35 drug consuming caregivers (age: 18 - 59 years) were analyzed for THCA-A and THC applying methanolic extraction and a fully validated LC-MS/MS method (Method 1) [4] as well as for THC applying alkaline hydrolyis and a HS-SPME-GC-MS method (Method 2) [5]. Furthermore, 30 hair samples were also analyzed for THCA-A and THC applying alkaline hydrolyis and a GC-MS method (Method 3) [3].

Results and discussion

Cannabinoid concentrations in children and adult hair

- THCA-A could be detected in all but one sample
- In 77 out of the 78 THCA-A positive cases the THCA-A concentration was higher than the THC concentration (Median: THCA-A : THC ratio 4.2)
- In 14 cases no THC could be detected despite THCA-A (median 63 pg/mg THCA-A)
- Trend: Higher concentrations in younger children (< 7 years)



THCA-A and THC concentration obtained by Method 1 as well as THCA-A to THC ratios in hair compared between adults and children, * and o indicate outliers.

Comparison within families

Significant higher THCA-A : THC ratios in hair of children in comparison to their adult caregivers when comparing within families (α = 0.042) (limited number due to data protection reasons)

- 6 cases: THCA-A : THC ratio higher than the ratio from the respective consuming caregiver
- 3 cases: Only THCA-A detectable in the hair samples of the children
- 1 case: THCA-A : THC ratio lower than the ratio from the respective consuming caregiver

Example of one family			
Age years	THCA-A pg/mg	THC pg/mg	THCA-A : THC
3	650	38	17.1
10	73	0	>7.3*
34	613	161	3.8
* Estimated using the LOD for THC (10 pg/mg)			

Possible explanation:

- Children hair: Dominating external contamination through contaminated fingers/surfaces.
- Adult hair: Combination of external contamination through contaminated fingers and through side-stream smoke elevating the THC concentration.

Impact of the applied methodology

Hair analysis for cannabinoids is further complicated by the fact that quite often alkaline hydrolysis is used as the method of sample preparation, leading to decarboxylation of THCA-A and therefore artificially elevating the THC concentration.

Method 2

Comparison of results obtained by Method 1 with the results obtained by Method 2			
	THC_{total}* Method 1 pg/mg	THC Method 2 pg/mg	THC_{total}* Method 1 : THC Method 2
Mean	603	585	1.48
Median	214	195	1.14
SD	960	862	1.20
Range	5.7 - 5200	11 - 4330	0.11 – 6.21
* THC_{total} = 314.47 x THCA-A/358:47 + THC			

The mean and the median of the concentrations of THC (Method 2) and of calculated THC_{total} (Method 1) are in relatively good agreement. However, focusing on paired values measured in the samples of the same individual indicates a high variation with the concentration ratio Method 1: Method 2 ranging from 0.11 to 6.21.

Method 3

Comparison of results obtained by Method 1 with the results obtained by Method 3					
	THCA-A Method 3 pg/mg	THC Method 3 pg/mg	THC_{total}* Method 1 pg/mg	THC_{total}* Method 3 pg/mg	Ratio Method 1 : Method 3
Mean	65.8	373	622	445	1.48
Median	45.8	157	328	212	1.49
SD	82.4	615	915	679	0.56
Range	0 - 419	0 - 2716	30 - 3956	19 - 2881	0.71 – 3.27
* THC_{total} = 314.47 x THCA-A/358:47 + THC					

Despite 10 min treatment in 1 N NaOH at 95 °C and heating during derivatization an essential part of THCA-A remains undecomposed. Generally, this can be an essential source of error in determination of THC in hair by alkaline hydrolysis.

Consequence of applying different methods

Example from an authentic sample:

Methanolic, LC-MS/MS (Method 1): THCA-A: 130 pg/mg & THC: n.d. (< LOD)

NaOH, LLE, GC-MS (Method 3): THCA-A: 46 pg/mg & THC: 65 pg/mg

Recommended cut-off for THC: 20 pg/mg (German driving license re-granting guidelines); 50 pg/mg (Society of Hair Testing)

Conclusion

The results show that the major part of the cannabinoids detected in the hair samples from children arose from an external contamination through 'passive' transfer by e.g. contaminated hands or surfaces and not from inhalation or deposition of sidestream smoke. Regarding the interpretation of hair samples in particular from young children, it has to be carefully evaluated if positive THC findings result from a smoke exposure or solely from external contamination without smoke exposure. The higher THCA-A : THC ratio in hair of children in comparison to their adult caregivers within families indicates dominating incorporation from contaminated hands and surfaces. Furthermore, it could be shown that the analytical results of hair analysis for cannabinoids strongly depend on the applied methodology mainly because of artifactual decarboxylation of THCA-A which leads to elevated THC concentrations. Therefore, it has to be questioned whether analyzing hair samples for THC using alkaline hydrolysis is adequate in hair analysis for cannabinoids.

[1] Moosmann et. al. Hair analysis for THCA-A, THC and CBN after handling of cannabis plant material. 51st TIAFT meeting Funchal, Madeira **2013**
[2] Moosmann et. al. Hair analysis for THCA-A, THC and CBN after passive in vivo exposure to marijuana smoke. Drug Test Anal 6 1-2 **2014**
[3] Auwärter et al. Hair analysis for Δ9-tetrahydrocannabinolic acid A—New insights into the mechanism of drug incorporation of cannabinoids into hair. Forensic Sci Int 196 10-13 **2010**
[4] Roth et. al. Development and validation of an LC-MS/MS method for quantification of Δ9-tetrahydrocannabinolic acid A (THCA-A), THC, CBN and CBD in hair. J Mass Spectrom 48: 2, **2013**
[5] Nadulski et. al. Simple ans sensitive determination of Δ9-tetrahydrocannabinol, cannabidiol and cannbinol in hair by combined silylation, headspace solid phase microextraction and gas chromatography-mass spectrometry J Chromatogr B 846 1-2 **2007**

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