Khat abuse as risk factor for development of psychotic symptoms. A feasibility study for further genetic epidemiological studies on addiction of khat

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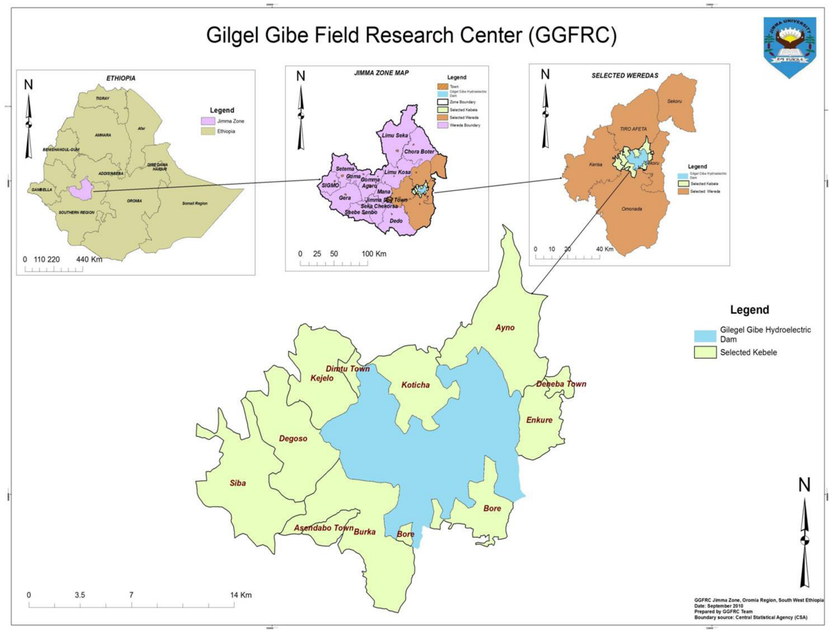
Abstract

Khat is a plant with a natural distribution limited to East Africa and countries on the Arabian Peninsula. Khat leaves are chewed for their stimulant and euphoric effects. Studies of the chemical constituents of khat have revealed that it contains different alkaloids such as norephedrine which have CNS stimulating effects. The psychotropic effects of khat are caused by the amphetamine-like compounds, which influence the dopaminergic pathways. Khat use can be considered as a lifestyle covering the spectrum from nonproblematic use to problematic use and dependence.

Introduction

The Jimma University in southwestern Ethiopia has a unique health and demographic surveillance system called „Gilgel Gibe Field Research Center" (GGFRC) with a catchment area of about 50.000 people. In this setting, we studied the effect of khat use as risk factor for the development and the stability of psychotic symptoms as well as of symptoms of common mental disorders among young men in the community. Furthermore, we wanted to demonstrate the reliability and validity of research methods that are necessary for future genetic epidemiological studies, i.e. the validity and reliability of pharmacological screening tests as well as assessments performed by trained local interviewers. Our study was the first psychopharmacological study in cooperation between the LMU in Munich, Germany and the JU in Ethiopia. Under suboptimal conditions (high temperatures, limited refrigeration options, lack of infrastructure), we collected biological samples and analyzed them on site.



Design

In this prospective study, trained local interviewers screened a representative cohort (N=852) of young men twice within a period of six months (T1, T3) to determine the presence and stability of distinct psychiatric symptoms. As part of the screening, urine samples were collected (T1, N=852) and analyzed for khat alkaloids by immunoassay tests for amphetamine. In a clinical validation interview (T2, N=126), to be conducted in a short period (1 – 3 days) after T1, a psychiatrist or mental health specialist reassessed the psychiatric symptom presentation in a randomly selected subgroup of 126 individuals of those persons who had been screened at T1. The validation study took also urine of this subgroup in order to validate the urine screening by a more extensive analysis of khat alkaloids (HPLC).

Methods

In our study, urine samples were collected randomly from khat chewers living in the Jimma zone of the GGFRC. During sample collection, samples were coded to facilitate ease of identification and the samples were transported to the study laboratory by a cold chain system preserving the integrity of the sample. After they had reached the study laboratory, samples were stored in refrigerators (2-8°C). To test the feasibility of a further genetic project, we collected blood samples as well. Blood samples were transported to the JU und stored in refrigerators (-80 °C) The average time from sample collection in the GGFRC until arrival at JU was 299.24 min. (5 h) (min. 95 min., max. 495 min). For DNA extraction a new method was established at the JU.

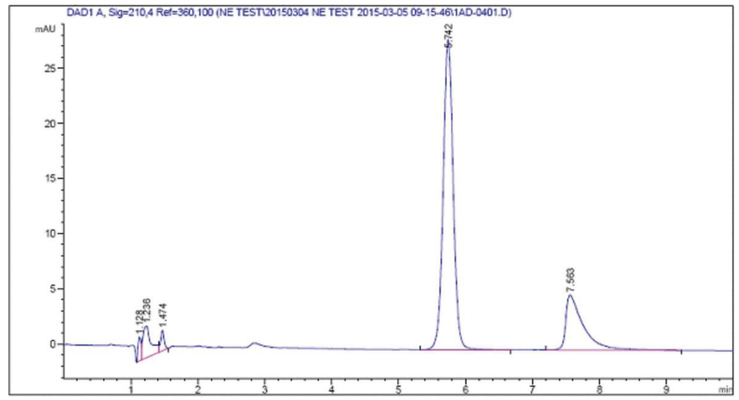


Figure 1. Chromatogram of norephedrine (NE) extracted from Recatol capsules

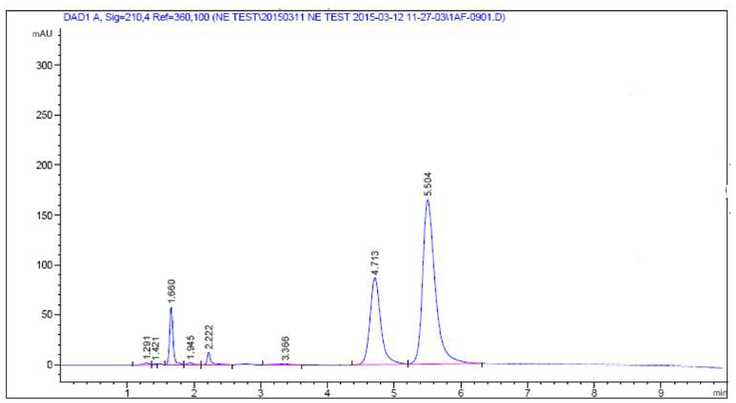
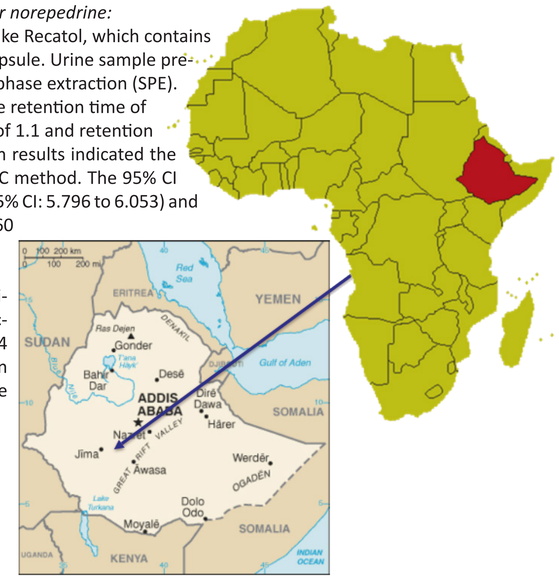


Figure 2. Chromatogram of norephedrine (NE) extracted from urine sample

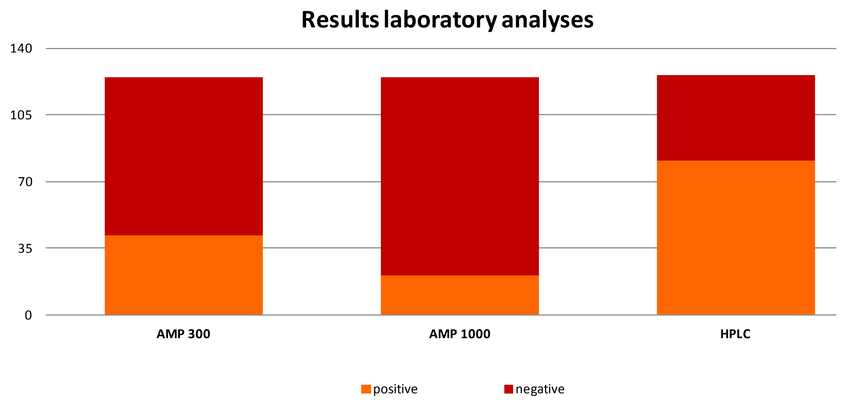
Design of the study	T1 Screening	T2 Validation interview	T3 Re-Screening
Performed by:	Local interviewers	Psychiatrist or mental health Specialist	Local interviewers
N: Men 18-30 years of age	N = 852	N = 126	N = 852
Sampling: Multi-stage stratified random sampling	Rural-urban,existing epidemiological cluster, Household, Individual	Randomly selected subgroup of T1; 50 with psychotic symptom presentation, 50 without	Re-assessment of all respondents from T1
Interview-based measures	CIDI	BPRS	CIDI
Biological measures	Urine: Khat alkaloids (amphetamine tests)	Urine: Khat alkaloids (HPLC for NE) Blood: DNA Extraction	Urine: Khat alkaloids (amphetamine tests)
Additional measures	1. Transport conditions, (storage, refrigeration) 2. Time until samples reach laboratory	1. Transport conditions, (storage, refrigeration) 2. Time until samples reach Laboratory	1. Transport conditions, (storage, refrigeration) 2. Time until samples reach laboratory

HPLC analyses of urine samples for norepedrine:
As reference, we used standards like Recatol, which contains about 50 mg norephedrine per capsule. Urine sample preparations followed by using solid phase extraction (SPE). Norephedrine elutes at an average retention time of 5.7 min with peak asymmetry As of 1.1 and retention factor k' of 4.2. Method validation results indicated the fitness-for-use of the applied HPLC method. The 95% CI for the regression slope = 5.925 (95% CI: 5.796 to 6.053) and y-intercept = 3.476 (95% CI: -25.960 to 32.913) together with r2 value of 0.999 and ANOVA F-value of 21548 proved a strong positive linear relationship. The limit of detection (LOD) for this method was 0.04 µg/ml and limit of quantification 0.14 µg/ml. This shows that the method is suited for both qualita tive and quantitative analysis.



Results

A total of 126 urine samples were extracted by using solid-phase extraction (SPE) apparatus and analyzed by using HPLC. The results indicated that 81 (64.3%) were positive and the remaining 45 (35.7%) samples were negative for norephedrine. For positive urine samples, the norephedrine content ranges from 2.3 to 161.1 µg/ml, with an average norephedrine content of 36.7 µg/ml. Compared with the immunoassay tests for amphetamine the HPLC was more sensitive: AMP 300 positive for 42 (33,3%) and negative for 83 (66,7 %); AMP 1000 positive for 21 (16,6%) and negative for 104 (83,4 %). In a total of 124 blood samples DNA were extracted by using DNA Blood10k Kits. The average DNA content in 10 samples were 243,1 ng/µl (content range from 143 to 352 ng/µl).



Future plans

Our project can be seen as a pilot and feasibility study to prepare a comprehensive population-based genetico-epidemiological study on various gene-environment interactions that should be carried out in the very next future. The infrastructure of GGFRC offers us a unique opportunity to build collectives of multiple-thousand people in a shortest period of time and to perform genetic studies as they have not yet been taken in Africa in this form so far. The extensive epidemiological registration of a population of 50.000 people, the stable population structure, and the quite stable environment, such as the urban and rural way of life with all its characteristics of an African country provide ideal conditions for this. The population is ideally suited to study the impact of polygenic risk profiles of various psychiatric disorders on behavioral traits and their interaction with environment.