

High prevalence of immunoglobulin E (IgE) sensitization among sisal (*Agave sisalana*) processing workers in Tanzania

Running title: IgE sensitization among sisal processors

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Abstract.

Purpose: Allergic sensitization among workers exposed to sisal is scarcely documented. We examined whether sisal processing is associated with IgE sensitization and its relationship to the prevalence of respiratory symptoms among Tanzanian processors.

Methods: 138 sisal exposed workers and 78 non-exposed controls were skin prick tested (SPT) using dry sisal extract and fresh sisal sap. Sera from a subset of 43 participants were analyzed for total and sisal specific IgE. SPT wheal size, prevalence of positive SPTs and adjusted relative risk (RR) for sisal sensitization were determined and compared between exposed and controls. Prevalences for respiratory symptoms were compared between sensitized and non-sensitized sisal workers.

Results: Significantly higher prevalence of positive SPTs to sisal was found among 74 % of sisal workers compared to 17 % among controls. Compared to controls, the RR of sensitization to sisal was 4 times (95 % CI; 2.4–6.7) among exposed workers. All exposed workers had elevated IgE levels (>100kU/L) and 27 % of tested sera had elevated sisal specific IgE. A high prevalence of respiratory symptoms was found in both sensitized and non-sensitized sisal workers

Conclusion: Sisal processing was associated with increased risk of IgE sensitization, but its clinical implication was not obvious.

Introduction

Several components of organic dust are considered to be allergens [28]. In addition to non-specific irritation in the airways, exposure to aero-allergens in agricultural populations may cause allergic inflammatory responses [2, 8]. Work-related allergies to airborne organic dusts have been reported in several groups of workers [1, 3, 9, 12, 15, 29, 35].

Episodic symptoms of running nose, redness and itching eyes, sneezing, wheezing and dyspnoea may represent allergic responses triggered by inhalation of aero-allergens [8]. In work-settings such allergic symptoms are likely to occur within 4 hours of starting the work shift, they are easily recognized among atopic individuals and are often linked to type 1 allergy. Mast cell degranulation and release of histamine and other inflammatory mediators are important components in type 1 allergic mechanisms [8]. Previous studies have described histamine releasing properties of sisal [24] and broncho-constrictive effects of histamine [5, 21]. Thus, sisal exposure might be involved in the aetiology of respiratory health effects. Recently, significantly higher prevalence of sneezing, running nose, and stuffy nose were reported among sisal workers compared to controls [16]. However, documentation of occupational allergic sensitization of workers exposed to sisal is scarce [31, 34].

Sisal, a natural fibre used for making ropes, carpets, paper and reinforcement material [19], is increasingly becoming one of the major agricultural export products of Tanzania [27], requiring a large labor force on the plantations and in sisal processing. The sisal workers are exposed to a large variety of aero-allergens and other organic particles which may have detrimental effects on their health. Knowledge of atopic status and sisal sensitization among the workers will help in planning and implementing health

surveillance and preventive measures in this industry. This paper examines whether sisal processing is associated with IgE sensitization and explores its relationship to the prevalence of respiratory symptoms among sisal processors in Tanzania.

Material and methods

Study design and participants

A cross-sectional study was conducted between June and October 2006. From a previous study on respiratory symptoms [16], all enrolled African men from brushing (n = 72) and decortication departments (n = 93) of six sisal processing factories were invited to constitute a sisal-exposed group. The workers in the decortication department work with raw sisal leaves and brushing workers handle dried decorticated sisal fibres. The control group comprised 80 African men who had never worked with sisal. They were enrolled from all available, healthy and willing guards, cleaners, car drivers, mechanics, salesmen and office clerks at an occupational health clinic situated about 120 kilometres from the nearest sisal estate. The distribution of the participants is detailed in figure 1 below. The purpose and methods of the study and the right to voluntary participation were clearly explained to all study participants, who also gave their written consents. The survey obtained ethical clearance from both Norwegian and Tanzanian medical research ethics authorities.

Figure 1

Questionnaires and interviews

For the exposed group, information on general demographics (age, height, weight and level of education), past respiratory illnesses (pneumonia, bronchitis and asthma and/or allergy), smoking habits (ever and current smoking), duration of employment in sisal

production and data on acute (92 workers) and chronic (137 workers) respiratory symptoms were retrieved from previous studies [16, 17].

Due to practical constraints we were not able to collect data on respiratory symptoms from the urban based control group. All those invited were asked whether they had ever worked in sisal factories and if they had used antihistamines 72 hours prior to the interview (exclusion criteria), then information on the participants age, educational level and smoking habits (ever smoking and current smoking) were recorded.

Skin prick tests

Fresh Sisal sap (FSS) was made in each estate from fresh cut *Agave sisalana* leaves. Every skin testing day a fresh leaf was thoroughly washed in running water, then crushed and squeezed. The green sap obtained was filtered into a sterile syringe and applied to the skin without any further dilution.

Dry Sisal extract (DSE) was prepared by soaking small pieces of dry fibres collected from the brushing machines in a bottle containing sterile physiological saline at ambient temperature at the ratio 1:1 volume/volume for 3–6 hours, with occasional mixing. The sisal saline mixture was filtered into a sterile syringe and as with FSS, new DSE was prepared in each estate before skin prick testing.

Two common allergens including commercially available extracts of timothy pollen (*Phleum pratense*; *TGP*) and house dust mite (*Dermatophagoides pteronyssinus*; *HDM* (-pilot tested on 13 sisal workers) were also tested. For positive and negative controls histamine 10 mg/ml and a diluent (ALK-Abelló, Hørsholm, Denmark), were used. All SPTs were performed in accordance with recommendations by the European Academy of Allergology and Clinical Immunology [10]. The SPT was considered positive if the

mean diameters of the duplicate wheals were 3 mm or greater than that of the negative controls. All SPTs were performed by the first author. During analysis two control participants and one decortication worker were excluded due to use of antihistamines. The mean diameter of 50 randomly selected histamine wheal duplicates was 5.40 mm with a coefficient of variation (CV) estimated from the differences between the duplicates of 17 %.

IgE and IgE antibodies

Three sisal estates located within a 4 hours drive from Dar es Salaam were visited on the same day for collection of blood samples from all workers available at the time of the visit (Figure 1). Collected blood samples were immediately stored in a cold container and sent to the Tanzania Occupational Health Services (TOHS) Clinic Laboratory in Dar es Salaam where serum was extracted. Blood samples from the controls were collected at TOHS after SPT. Only 8 control participants were willing to give blood samples, their main reasons for refusal being religious or fear of the procedure. Serum samples were transported in an ice packed cooler to the Laboratory of Clinical Biochemistry, Haukeland University Hospital, Bergen, in Norway for analysis. Serum IgE measurements were performed by using the ImmunoCAP-FEIA system, (Phadia, Uppsala, Sweden) assaying total IgE and PhadiatopTM (a panel of inhalant allergens including house dust mites and pollen from timothy). Total serum IgE ≥ 100 kU/L were considered to be elevated [31]. and PhadiatopTM results were interpreted positive if ≥ 0.35 kU/L.

Enzyme-linked immunosorbent assay (ELISA)

Sisal extracts (*SE*) were prepared by homogenization and suspension of a piece of fresh sisal leaf in 50 mM (millimolar) NH_4HCO_3 (pH 8.0) to a volume of 100 ml. The mixtures were incubated overnight at 4 °C and dialyzed (cut-off 8000 Units) for 48 hours. The extracts were then lyophilized and stored at minus 20 °C until used.

ELISA test as described by Holen et al. [13] was employed to determine sisal IgE reactivity. Serial concentrations from 0.0, 0.1 to 4.0 µg (micrograms) of sisal extract were tested as coating allergen by use of serum pool of the same 7 sisal allergic subjects as used in SDS-Immunoblots. Sisal extract cut off point of 0.5 µg was found to be the optimal concentration for coating of plates. Thus 96-well microtiter plates (Microtiter plates, Dynatech Laboratories Inc., Chantilly, VA, USA) were coated with 0.5 µg sisal extract (*SE*), dissolved in 100 µl (microliter) of 100 mM sodium carbonate, pH 9.6 and incubated overnight at 4 °C

The plates were washed with Tris buffer pH 7.4 containing 0.05 % Tween-20 (TBS Tween), then 100 µl / well of serum were added, and incubated overnight at 4 °C. After washing with TBS Tween, anti-human IgE alkaline Phosphatase conjugate (Sigma; 1:1000 dilution) was added 100 µl / well and incubated for 2 hours at room temperature. After another wash, the colour reaction was developed with 100µl / well of Tris buffer pH 9.5 containing 1 mg/ml *p*-nitrophenylphosphate (Sigma). Absorbance was read at 405 nm after 10 minutes in an ELISA reader.

Sisal protein separation and immunoblotting.

Sisal extracts (*SE*) made from fresh sisal leaf were separated by Sodium dodecyl sulphate-polyacrylamide gel electrophoresis (SDS-PAGE) according to the procedure by Laemmli [18]. The samples were resolved in a 12 % gel at 200 V and proteins were

visualized by Coomassie brilliant blue R-250 staining (Sigma Chemical Co., St. Louis, MO, USA). Immunoblotting was performed by transferring the proteins onto nitrocellulose membranes (0.45 µm, Schleicher and Schüell, Dassel, Germany) for 1 h at 100 V in a mini trans-blot cell (BIO-RAD, Richmond, CA, USA). The blots were then incubated overnight with sisal allergic patients' serum pool for IgE binding. After specific IgE binding, the colours were developed using SIGMA FAST™ BCIP/NBT tablets (Sigma).

Statistical methods

Before statistical analysis SPT wheals below 0.5 mm were assigned $0.5/\sqrt{2}$ (i.e.0.35 mm) value according to Hornung *et al* [13]. Sensitization to sisal was defined as positive SPTs to fresh sisal sap and/or to dry sisal extract in addition to those with positive ELISA despite having negative SPTs.

To test differences between sisal workers and controls, Pearson's Chi-square test, and where expected values were less than 5, Fisher's exact tests were used to compare categorical variables of smoking habits, education status, past respiratory illnesses, acute and chronic respiratory symptoms, SPTs and Phadiatop™ positivity, total and specific IgE levels. Mean differences for diameters of SPTs wheals, Phadiatop™ reaction, total and sisal specific IgE results were tested by independent t-test, which was also used to test the differences for continuous variables of age, body mass index and years in the current job title. Correlation between mean SPT wheal diameters and other continuous variables were estimated by Pearson's correlations. All continuous variables except for age were log transformed.

Log-binomial regression models were developed to estimate relative risk for sensitization to sisal among the exposed compared to controls adjusting for age and ever

smoking. For sisal workers, relative risks were estimated for acute and chronic respiratory symptoms among sensitized compared to non-sensitized workers, adjusting for age, past respiratory illnesses and either current smoking for acute respiratory symptoms or ever smoking for chronic symptoms. The data were analyzed using SPSS version 13 for Windows (Chicago, IL, USA) and STATA version 9.2. Statistical significance level was set to 0.05.

Results

Sisal workers were significantly older and more likely to be smokers than controls (Table 1). Exposed workers were also less likely to have attained more than 7 years of education and had worked in their current jobs for a mean of 13 years.

Table 1

Prevalence of positive SPTs to sisal and common allergens

The mean wheal diameters of SPT reactions to sisal were largest for FSS among decorticators (3.2 mm) and smallest for DSE among controls (0.8 mm). The corresponding prevalence's of positive SPTs were for decorticators (60 %) and controls

Table 2

(2.6 %) (Table 2). Significantly higher prevalence of positive SPTs and significantly larger mean wheal diameters were found among exposed workers than among controls (Table 2). Prevalence of positive SPT to sisal was significantly higher among ever smokers ($p < 0.01$) and current smokers ($p = 0.04$) compared to non-smokers (not shown in tables). Age and smoking adjusted relative risk for sensitization to sisal was 4 times among sisal workers compared to controls (Table 3).

Table 3

Thirty percent of sisal workers were sensitive to grass pollen compared to 1.4 % among controls while 9/13 (69%) tested sisal workers also showed positive SPTs to house dust mites. SPT wheal sizes for FSS and DSE correlated positively with timothy pollen

wheals ($r = 0.3$; $P < 0.001$, respectively), and age ($r = 0.1$; $P < 0.05$) and ($r = 0.2$; $P < 0.01$), respectively. All study participants showed a reaction to histamine (Mean, 5.7; range, 2 – 9.5 mm) and none to the diluent (negative control).

Specific IgE to sisal:

Using sisal extract (SE) to run ELISA, mean optical density (OD) were highest for decorticators (Table 4). Overall, 11 of 41 subjects (27 %) had elevated specific IgE levels against sisal extract ($OD < LOD - 2.6$). The prevalence of sensitization to sisal did not differ among the study groups. All ELISA positive subjects had positive SPTs.

Table 4

IgE and PhadiatopTM

Total IgE levels were higher among exposed workers than among controls (Table 4), but the difference was only significant between decorticators and controls. All exposed workers and all but one control had elevated ($> 100kU/L$) IgE levels. Five sisal workers had $> 5000 kU/L$ total IgE compared to none in the controls. 32 of 43 (78 %) sampled subjects had at least one positive specific IgE to the tested allergens. No significant differences were found between the study groups for PhadiatopTM results or prevalence of elevated serum IgE. Total IgE levels showed positive correlations with PhadiatopTM ($r = 0.7$; $p < 0.001$) and FSS wheals sizes ($r = 0.4$; $P < 0.05$).

SDS-PAGE results

Sisal extract analysis by SDS-PAGE and immunoblotting was done using a serum-pool of 7 subjects positive to sisal. Two IgE binding protein bands were detected at about 45 kDa (Figure 2).

Figure 2

Sisal sensitization and respiratory symptoms

Acute rhinitis was reported by 71%, acute lower respiratory symptoms by 74 % and chronic respiratory symptoms by 52 % of the sensitized sisal workers (Table 3). Adjusted relative risks among sensitized compared to non-sensitized sisal workers were for acute rhinitis (RR; 1.05), acute lower airways symptoms (RR; 0.93) and chronic respiratory symptoms (RR; 1.12) (Table 3)

Discussion

While previous studies have demonstrated acute and chronic respiratory effects among sisal workers in Tanzania [16, 17, 23], immunological reactions have not been investigated. In this study, four times as many sisal workers were IgE sensitized to sisal compared to controls. Elevated specific IgE to sisal was observed among 27 % of the tested subset, all of whom had positive SPT to sisal.

Our overall findings of SPT wheal diameters of 0.35 -7.50 (mm) following subcutaneous skin pricks with sisal extracts are somehow lower than the finding reported many years ago (1955), among sisal factory workers in Kenya [31]. Strong skin reactions (mean indurations; >10mm) were reported among 105 male sisal factory workers, following an intra-cutaneous injections with sisal extract made from rafters in the sisal carding room [31]. The intra-cutaneous skin prick method used by Stott may explain the moderately higher skin reactivity in the Kenyan study. In his study however, Stott did not observe any differences in skin reaction to sisal extract between workers who had never worked with sisal, or had worked for less than 6 months in the sisal carding room and those who had more than 6 month in the carding sisal room. On another hand, our findings of 36 % and 42 % sensitization to dry sisal extract among sisal decorticators and brushing workers respectively, is about four times higher than the

10 % prevalence reported by Zuskin *et al.*[34] among female textile workers in Croatia. However, work processes and extract preparation methods used in the Croatian and Kenyan studies were also different from our study. In these two previous studies [31, 34], processed sisal was presumably used to make textile, and dust from the work rooms was used to prepare the extract for testing. We used both fresh and dry non-processed sisal fibre extracts and found a higher prevalence of sensitization to fresh sisal sap than to dry sisal extract. These findings suggest that fresh sisal may contain more of the allergenic and/or irritating substance(s), which progressively becomes reduced during processing of the fibres. Constituents in sisal in Croatia and Tanzania may also be different. Further analysis will be needed to identify allergenic molecules within the detected protein bands in sisal.

High prevalence of allergic sensitization has been reported in other studies of organic dusts. A study among hemp and flax textile workers in Croatia [37] showed a prevalence of positive SPT of 48 % to flax extract, 41 % to hemp dust extracts from the combing machines, and 64 % to a combined extract compared to 5–21 % among controls. A 34.9 % prevalence of positive SPTs to coffee extract was reported among coffee workers in Uganda as compared to 7 % in controls [29] and to 24 % among coffee processors in Croatia [35]. Similarly, in a group of 24 cotton textile workers in Yugoslavia, Zuskin *et al.* [35, 36] found a 33.3 % prevalence of sensitization to cotton seed/dust extract.

Our findings of 71 % of acute rhinitis among sensitized workers is higher than the 16–50% observed among workers in a tea packing factory [1], or 5–42 % among Norwegian farmers [22]. As opposed to the lack of clear association between sensitization to sisal and respiratory symptoms found in our study, a significantly higher prevalence of chronic respiratory symptoms was reported among hemp sensitized

workers than among non-sensitized workers in Croatia [37]. However, similar lack of associations has been reported in relation to other organic dusts [15, 35, 36]. Furthermore our finding of high prevalences of respiratory symptoms in both sensitized and non-sensitized sisal workers may suggest a co-existence of several causative mechanisms [15].

In addition to occupational exposures to allergens, the presence and exposure to local aeroallergens is an important factor [4]. In our study, almost a third of the sisal workers showed positive reactions to timothy grass pollen. In addition, 69 % (9/13 of sisal processors) had positive SPT to house dust mite, slightly higher than the 40 % (8/20 found by Zuskin *et al.* [34] among sisal textile workers in Croatia and the 48.7 % reported by Sunyer *et al.* [32] among women in Tanzania.

The prevalence of atopy assessed on the basis of elevated total IgE and positive PhadiatopTM was high in our study. As opposed to 10 % of sisal workers in the Croatian study [34], 35.7 % among hemp processing workers [37] or 5/8 workers (62 %) observed among sensitized cotton workers [36], all our sisal workers had elevated serum IgE levels. Comparative data on specific and total IgE in African populations are scarce [6, 30]. However, our 100 % prevalence of elevated serum IgE and 77 % prevalence of specific IgE to common allergens among sisal workers are similar to the 95.7 %, and 73.3 %, respectively, among women in a semirural area of Tanzania [32].

In addition to pre-existing atopic status, several other factors influence immunological responses [4, 26, 33]. Parasitic infections may potentiate the allergic response to other allergens and the production of parasite specific IgE-antibodies may lead to increased total IgE [7, 11]. Tanzania has a typical tropical environment where several infections may co-exist. This could explain our findings of generally higher serum IgE levels. However, our blood samples for immunological analysis might have been too few to

find significant differences between the groups. IgE sensitization may also be influenced by age and smoking habits [4, 25, 26]. In the current study, smokers had higher prevalence of positive SPT and a positive correlation was found between age and skin sensitization. Sensitization to sisal was therefore adjusted for both age and smoking.

Our controls live in Dar es Salaam city. The difference in geographical location makes them less likely to be exposed to sisal than workers in the sisal estates. They were thus assumed to be an appropriate control group for studying differences in sensitization to sisal despite their presumed higher socioeconomic status [20]. Availability of information on respiratory symptoms from this group would have been an advantage, but unfortunately, this was not practically possible.

As the sisal extract antigen, may be considered not to be highly purified and/or enriched, the use of conventional ELISA immunoassay plates may therefore have underestimated the prevalence of subjects with elevated IgE to sisal. However, the method was used both for the exposed and the controls, showing high prevalence of elevated IgE levels in both groups. Future studies should explore the use of other methods.

Using a cross-sectional design, we are unable to control for the healthy worker effect and /or separate the temporal relationship between sensitization to sisal and possible health outcomes..

Conclusions

Work in sisal processing is associated with an increased risk of IgE sensitization. Both immunological and non-immunological mechanisms (mechanical irritations, and/or local toxicity) may co-exist in relation to exposure to sisal. In this study, sensitization to

sisal was not clearly associated with self-reported airway symptoms. Larger studies and further analysis to identify and characterize the sisal allergen(s) may be beneficial.

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Table 1: Characteristics of the study population, grouped into departments of decortication, brushing and controls

Variables	Decorticators (n = 77)			Brushing (n = 61)			All exposed ^d (n = 138)			Controls (n = 78)	
	AM	(range)	P ^a	AM	(range)	P ^b	AM	(range)	P ^c	AM	(range)
Age (yrs)	46	(19–85)	<0.001	49	(18–82)	<0.001	47	(18–85)	<0.001	35	(19–65) ^e
BMI (%)	20	(16–29)	-	20	(16–27)	-	20	(16–29)	-		na
Years in current job	11	(<1–56)	-	14	(<1–49)	-	13	(<1–56)	-		na
	n	(%)		n	(%)		n	(%)		n	(%)
<i>Smoking habits</i>											
Ever smoking	56	(73)	<0.001	43	(71)	<0.001	99	(71)	<0.001	23	(30)
Current smoking	43	(56)	<0.001	31	(51)	<0.001	74	(54)	<0.001	20	(26)
<i>Education years</i>											
None	22	(29)		16	(26)		38	(28)		4	(5.1)
1 to 7 years	54	(70)	<0.001	42	(69)	<0.001	97	(70)	<0.001	29	(37)
> 7 years	1	(1.3)		3	(4.9)		4	(2.9)		45	(58)
Past respiratory illnesses	36	(47)	-	23	(38)	-	59	(43)	-		na
<i>Current Respiratory symptoms</i>											
Acute rhinitis ^f	29	(62)	-	36	(80)	-	65	(71)	-		na
Acute lower airway symptoms ^f	33	(70)	-	37	(82)	-	69	(75)	-		na
Chronic respiratory symptoms ^g	33	(43)	-	35	(57)	-	68	(50)	-		na

Comparisons; ^adecorticators vs. controls; ^bbrushing vs. controls; ^call exposed vs. controls; AM - arithmetic mean; BMI - Body mass index

^ddecorticators plus brushing; ^edata on age available for 71 of 78 controls; na - data not available;

^ffor acute symptoms data were available for 92 sisal workers (n = 47; decorticators and n = 45; brushing)

^gfor chronic symptoms data were available for 137 (n = 76; decorticators and n = 61; brushing)

Table 2: Results from Skin prick tests (SPT) among all examined sisal workers and controls

	Decorticators (N = 77)			Brushing (N = 61)			All exposed (N = 138)			Controls (N = 78)	
	AM(ME)	Range	p ^a	AM(ME)	Range	p ^b	AM(ME)	Range	p ^c	AM(ME)	Range
Histamine	5.9 (6.0)	(3.3–8.4)	<0.001	6.0 (5.8)	(4.5–9.5)	<0.001	5.9 (5.8)	(3.3–9.5)	<0.001	5.3 (5.3)	(2.1–6.9)
Grass pollen ^g	2.2 (2.4)	(0.3–5.3)	0.001	1.9 (1.8)	(0.3–5.4)	0.057	2.0 (2.2)	(0.3–5.4)	0.001	1.2 (1.1)	(0.3–3.0)
Dry sisal extract	2.5 (2.8)	(0.3–6.8)	<0.001	2.3 (2.6)	(0.3–5.3)	<0.001	2.4 (2.8)	(0.3–6.8)	<0.001	0.8 (0.3)	(0.3–3.4)
Fresh sisal sap	3.2 (3.1)	(0.3–7.1)	<0.001	3.0 (3.0)	(0.3–7.5)	<0.001	3.1 (3.1)	(0.3–7.5)	<0.001	1.5 (1.6)	(0.3–4.3)
<i>Prevalence's</i>	<i>n (%)</i>		<i>p^d</i>	<i>n (%)</i>		<i>p^e</i>	<i>n (%)</i>		<i>p^f</i>	<i>n (%)</i>	
Grass pollen ^g	24 (31)		<0.001	18 (30)		<0.001	42 (30)		<0.001	1 (1.4)	
Dry sisal extract	32 (42)		<0.001	22 (36)		<0.001	54 (39)		<0.001	2 (2.6)	
Fresh sisal sap	46 (60)		<0.001	35 (57)		<0.001	81 (59)		<0.001	12 (15)	
Dry/Fresh sisal	57 (74)		<0.001	43 (71)		<0.001	100 (73)		<0.001	13 (17)	

Key: AM - arithmetic mean; ME - median; OD - optical density

Independent t- tests; ^adecorticators vs. controls; ^bBrushing vs. controls; ^call exposed vs. controls;

Pearson chi-square or Fisher's exact tests; ^ddecorticators vs. controls; ^eBrushing vs. controls; ^fall exposed vs. controls

^gN for control =71; 7controls not tested for grass pollen.

Table 3: Relative Risk (RR) for sensitization to sisal^a among sisal workers compared to controls and RRs for self-reported respiratory symptoms^{b,c,d} among sensitized compared to non-sensitized sisal workers.

<i>Sensitization</i>	Groups	Prevalence of sensitization			RR _{adj} ^e	(95 % CI)
		<i>N</i>	<i>n</i>	(%)		
Sensitization to sisal ^a	Controls	78	13	(17)	Ref	-
	Sisal workers	138	100	(73)	4.00	(2.4–6.7)
Prevalence of respiratory symptoms						
<i>Respiratory Symptoms</i>		<i>N</i>	<i>n</i>	(%)	RR _{adj} ^f	(95 % CI)
Acute rhinitis ^b	Non sensitized	22	15	(68)	Ref	-
	Sisal sensitized	70	50	(71)	1.05	(0.7–1.5)
Acute low airway symptom ^c	Non sensitized	22	17	(77)	Ref	-
	Sisal sensitized	70	52	(74)	0.93	(0.7–1.2)
Chronic respiratory symptoms ^d	Non sensitized	38	17	(45)	Ref	-
	Sisal sensitized ^g	99	51	(52)	1.12	(0.8–1.6)

Key: N- number of responders; n -number sensitized or with symptoms accordingly

^aSensitization to sisal (positive SPT to fresh sisal sap and/or dry sisal extract + sisal IgE >0.1 OD)

^bAcute rhinitis ('yes' to either stuffy nose, running nose or sneezing during or after the work shift)

^cAcute lower respiratory symptoms ('yes' to either dry cough, productive cough, shortness of breath or wheezing during or after the shift)

^dChronic symptoms ('yes' to either chronic cough, cough with sputum, wheezing, dyspnoea or chest tightness)

^eRelative risk, adjusted for age and ever smoking

^fRelative risk; adjusted for age, smoking, and past respiratory illnesses

^gOne sensitized sisal worker had no data for chronic symptoms due to deafness

Table 4: Results of Sisal Specific IgE by ELISA (OD), Total IgE (kU/L) and Phadiatop™ (kUA/l) from 43 tested study participants

	Decorticators (N = 19)			Brushing (N = 16)			All exposed (N = 35)			Controls (N = 8)	
	AM(ME)	Range	p ^a	AM(ME)	Range	p ^b	AM(ME)	Range	p ^c	AM(ME)	Range
Specific Sisal IgE	0.3 (0.1)	(0.0–2.6)	0.28	0.1 (0.04)	(0.0–0.3)	0.86	0.2 (0.05)	(0.0–2.6)	0.56	0.1 (0.04)	(0.0–0.6)
Phadiatop™ ^g	5.7 (2.6)	(0.3– 41)	0.43	10 (3.2)	(0.2– 48)	0.67	7.8 (2.8)	(0.2– 48)	0.47	20 (6.4)	(0.3–88)
Total IgE	2230(2050)	(105–5000)	0.04	2450(2160)	(203–5000)	0.07	2230(2048)	(105–5000)	0.05	810 (710)	(65–1900)
<i>Prevalence's</i>	<i>n (%)</i>		<i>p^d</i>	<i>n (%)</i>		<i>p^e</i>	<i>n (%)</i>		<i>p^f</i>	<i>n (%)</i>	
Sisal IgE >0.1	5 (28)		1.00	4 (27)		1.00	9 (27)		1.00	2 (25)	
Phadiatop™>0.35 ^g	13 (69)		1.00	13 (87)		0.59	26 (77)		1.00	6 (75)	
Total IgE>100	19 (100)		0.30	16 (100)		0.33	35 (100)		0.19	7 (88)	

Key : AM - arithmetic mean; ME - median; OD - optical density

Independent t- tests; ^adecorticators vs. controls; ^bBrushing vs. controls; ^call exposed vs. controls;

Pearson chi-square or Fisher's exact tests; ^ddecorticators vs. controls; ^eBrushing vs. controls; ^fall exposed vs. controls;

^gTotal samples brushing was 15 (one sample from brushing not included due to insufficient sera)

Figure 1: Distribution of participants of IgE sensitization study among sisal processors

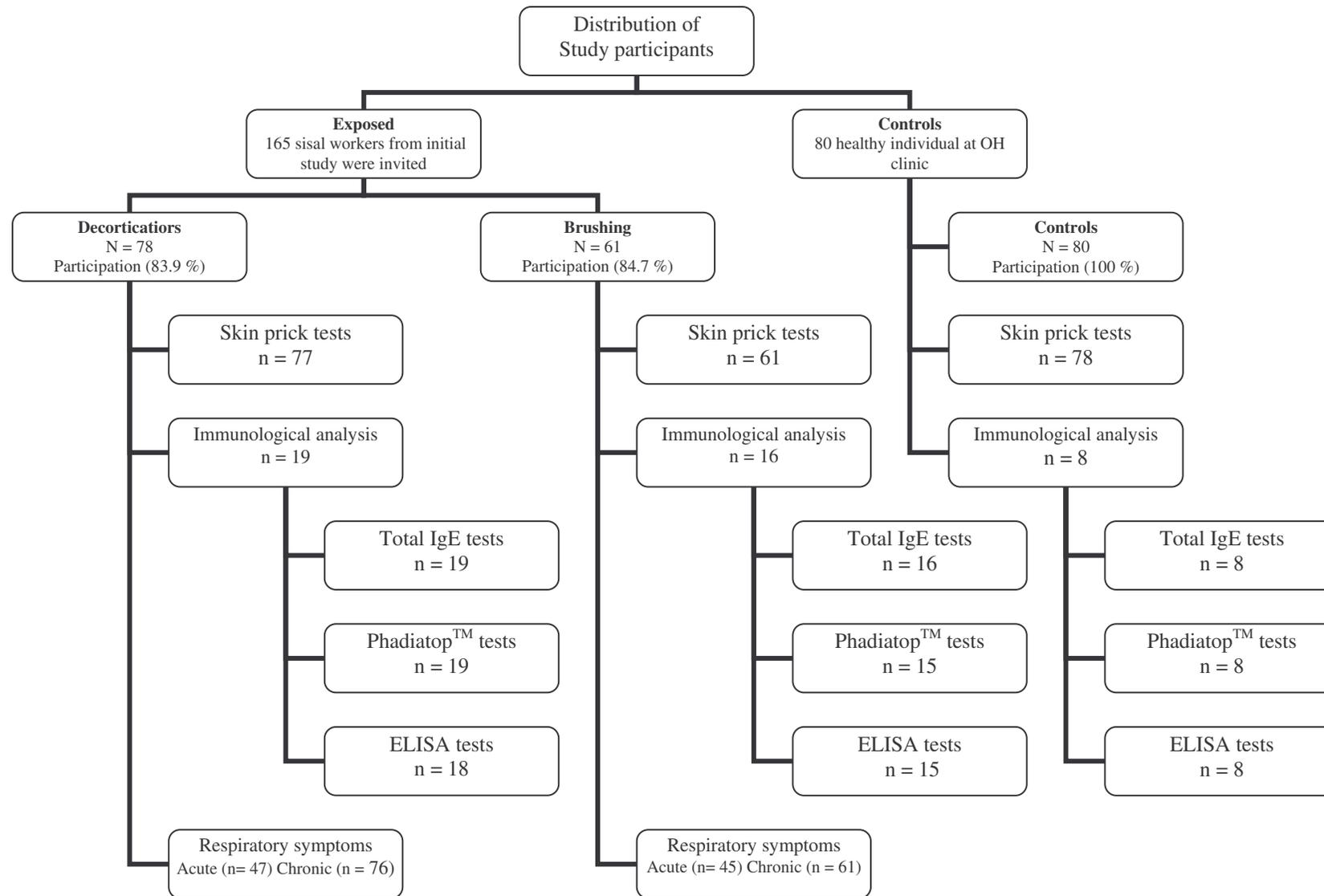
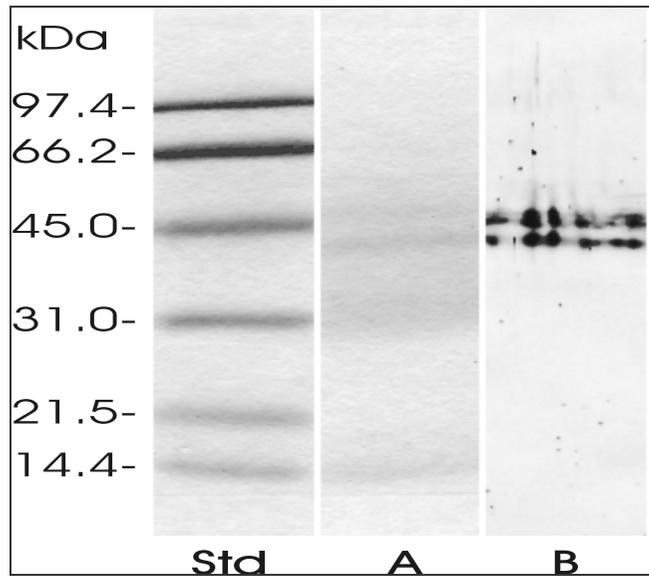


Figure 2: SDS-PAGE^a and immunoblots of sisal extract from fresh sisal leaf.



Key

^a(Sodium dodecyl sulphate-polyacrylamide gel electrophoresis method described by Laemmli et al. 1970)

Std - standard markers.

A - SDS-PAGE.

B - Immunoblots.