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Altaf, Quratul-ain Altaf; Ali, Asad; Piya, Milan K.; Raymond, Neil T.; Tahrani, Abd A.

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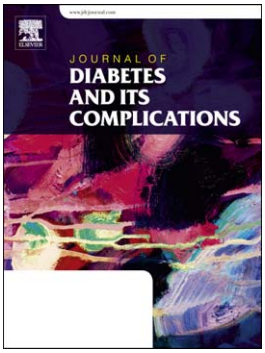

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Q.A. Altaf, Asad Ali, Milan K. Piya, Neil T. Raymond, Abd A. Tahrani

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The relationship between obstructive sleep apnoea and intra epidermal nerve fiber density, PARP activation and foot ulceration in patients with type 2 diabetes

Q A Altaf[1,2], Asad Ali[3], Milan K Piya[4,5], Neil T Raymond[6], Abd A Tahrani[1,2]

[1]Department of Diabetes and Endocrinology, Heart of England NHS Foundation Trust, Birmingham, UK

[2]Institute of Metabolism and Systems, College of Medical and Dental Sciences, University of Birmingham, Birmingham, UK

[3]Department of Respiratory Medicine, University Hospitals Coventry and Warwickshire NHS Trust, Coventry, UK

[4]Warwickshire Institute for Diabetes, Endocrinology and Metabolism, University Hospitals Coventry and Warwickshire NHS Trust, Coventry, UK

[5]Institute of Digital Health, Warwick Manufacturing Group, University of Warwick, Coventry, UK

[6]Independent Epidemiology and Statistical Consultant, Epidemiology, Research Design and Statistical Consulting (ERDASC), Leicestershire, UK.

Corresponding author

Dr Abd A Tahrani

Department of Diabetes and Endocrinology,

Birmingham Heartlands Hospital, Birmingham B9 5SS, UK

Tel: +44 (0)7801549960

E mail: abd.tahrani@nhs.net

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Abstract:

Background: Obstructive sleep apnoea (OSA) is associated with increased nitrosative stress, endothelial dysfunction, and peripheral neuropathy in patients with type 2 diabetes. We hypothesized that OSA is associated with Poly ADP ribose polymerase (PARP) activation, lower intra epidermal nerve fibre density (IENFD), and diabetic foot ulceration (DFU).

Methods: A cross-sectional study of adults with type 2 diabetes recruited from a secondary care hospital in the UK. OSA was assessed by multi-channel home-based cardio-respiratory device (Alice PDX, Philips Respironics). DPN was assessed using the Michigan Neuropathy Screening Instrument (MNSI). IENFD and % PAR stained nuclei were assessed using immunohistochemistry staining on skin biopsies. DFU was assessed based on MNSI.

Results: Skin biopsies and DFU data were available from 52 and 234 patients respectively. OSA was associated with lower IENFD (12.75±1.93 vs. 10.55±1.62 vs. 9.42±1.16 fibres/mm of epidermis for no OSA, mild OSA and moderate to severe OSA respectively, p<0.001). Following adjustment, mild (B=-2.19, p=0.002) and moderate to severe OSA (B=-3.45, p<0.001) were independently associated with IENFD. The apnoea hypopnea index (AHI) was associated with IENFD following adjustment (B=-2.45, p<0.001). AHI was associated with percentage of PAR stained nuclei following adjustment (B=13.67, p=0.025). DFU prevalence was greater in patients with OSA (7.1% vs. 28.1% vs. 26.2% for patients with no OSA, mild OSA and moderate to severe OSA respectively, p=0.001). Following adjustment, OSA was associated with DFU (OR 3.34, 95%CI 1.19-9.38, p=0.022).

Conclusions: OSA is associated with lower IENFD, PARP activation and DFU in patients with Type 2 diabetes. Our finding suggest that OSA is associated with small fiber neuropathy. PARP activation is a potential mechanisms linking OSA to DPN and endothelial dysfunction in patients with Type 2 diabetes. Whether OSA treatment will have a favorable impact on these parameters and DFU requires interventional studies.

Key words: foot ulceration, neuropathy, sleep apnoea, type 2 diabetes, intra epidermal nerve fibre density, PARP

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Introduction

Obstructive sleep apnoea (OSA) is very common in patients with Type 2 diabetes and is characterized by recurrent episodes of upper airway obstruction associated with recurrent oxygen desaturations, cyclical changes in blood pressure (BP), heart rate and intrathoracic pressure, as well as recurrent arousals from sleep and changes in sleep architecture (Tahrani et al., 2013b).

We have previously shown that OSA was associated with foot insensitivity (based on 10g monofilament test) and diabetic peripheral neuropathy (DPN)(based on the Michigan Neuropathy Screening Instrument MNSI) (Tahrani et al., 2012a). These assessments are largely based on large fiber neuropathy; while the relationship between OSA and small fiber neuropathy is unknown. Intra epidermal nerve fiber density (IENFD) is considered an accurate and reproducible measure of early diabetic neuropathy and the "gold standard" for small fiber neuropathy (England et al., 2009;Lauria et al., 2010;Malik et al., 2011). Hence we hypothesized that OSA was associated with lower IENFD in patients with Type 2 diabetes.

We have also shown previously that OSA was associated with impaired cutaneous microvascular function (assessed by Laser Speckle Contrast Imaging technique) and impaired responses to nitric oxide(Tahrani *et al.*, 2012a). OSA was associated with increased nitrosative stress and oxidative stress in patients with Type 2 diabetes which might have contributed to the associations between OSA and impaired microvascular function and DPN(Tahrani *et al.*, 2012a). Hyperglycemia-induced nitrosative and oxidative stress play an important role in the development of vascular disease in patients with diabetes, via the activation of Poly ADP ribose polymerase (PARP), the inhibition of Glyceraldehyde 3-phosphate dehydrogenase (GAPDH) and the activation of several pathways (such as the polyol pathway and advanced-glycation end product (AGE)) resulting in inflammation, endothelial dysfunction and microvascular disease(Brownlee, 2005;Tahrani et al., 2010). Hence, we hypothesized that OSA is associated with PARP activation in patients with Type 2 diabetes which can mediate the relationship between OSA and nitrosative stress and endothelial dysfunction, which we described previously (Tahrani *et al.*, 2012a).

PARP activation and oxidative stress have also been shown to play an important role in the pathogenesis of diabetic foot ulceration (DFU) (Tahrani et al., 2012b). Based on the association between OSA and DPN, abnormal 10g monofilament, oxidative and nitrosative stress and possibly PARP activation, we hypothesized that OSA is associated with DFU in patients with Type 2 diabetes.

Hence the primary aim of this study is to examine the relationship between OSA and IENFD and PARP activation in the skin of patients with Type 2 diabetes. A secondary aim is to explore whether there is a link between OSA and DFU in this study population.

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Methods:

We conducted an observational cross-sectional study in adults with Type 2 diabetes, the results of which have been published previously (Tahrani *et al.*, 2012a). Patients with respiratory disease (including pre-diagnosed OSA), end-stage renal disease or non-diabetic neuropathy (<1%) were excluded. Patients were recruited opportunistically from the out-patient diabetes departments of a UK hospital. Patients were approached in the waiting area before they have seen the clinicians and without any prior knowledge of their medical condition. We avoided any reference to snoring during the recruitment process. Consent was obtained and ethnicity self-determined in accordance with the UK decennial census by the study participants. The project was approved by the Warwickshire Research Ethics Committee (REC number 08/H1211/145) and all patients were consented accordingly.

DPN was assessed using the Michigan Neuropathy Screening Instrument (MNSI), a validated, 2-component tool designed to facilitate the diagnosis of DPN, which has been widely used (1988;1999;Boyraz and Saracoglu, 2010;Feldman et al., 1994a;Martin et al., 2006;Pambianco et al., 2011).The questionnaire component (MNSIq) comprises 15 questions seeking to characterize sensory disturbance, but also peripheral vascular disease and general asthenia (Feldman et al., 1994b). The examination component (MNSIe) comprises a limited foot inspection to identify deformity, skin abnormalities and ulceration, coupled with an assessment of vibratory perception at the great toe (measured using a 128 Hz tuning fork) and ankle tendon reflexes. DPN was diagnosed if the MNSI examination score was >2 and/or MNSI questionnaire score was ≥ 7 (Martin *et al.*, 2006;Pambianco *et al.*, 2011). Foot insensitivity to a 10-g monofilament (applied to 10 foot locations) was defined as < eight correct responses (Pambianco *et al.*, 2011).

OSA was assessed by a single overnight home-based cardio-respiratory sleep study using a portable multi-channel device (Alice PDX, Philips Respironics) and scored in accordance with the American Academy of Sleep Medicine guidelines (Iber C et al., 2007). An apnoea hypopnea index (AHI) ≥ 5 events/hour was consistent with the diagnosis of OSA (Epstein et al., 2009). Sleep studies with <4 hours of adequate recordings were repeated and excluded if the quality remained poor. All sleep studies were scored independently by two researchers.

All patients of the original study were approached to provide skin samples to assess IENFD and %PAR stained nuclei, and skin biopsies were performed on patients who consented. Skin biopsies were obtained from the mid-thigh level under local anesthetic and using aseptic techniques.

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IENFD was assessed as we described previously (Tahrani *et al.*, 2012b) and recommended by others (Lauria *et al.*, 2010). Four randomly selected 50 µm sections were immunohistochemically stained using a free floating protocol with rabbit anti-human polyclonal PGP9.5 antibody (Biogenesis 1:1200). Individual ENFs were counted as they passed through the basement membrane. Negative controls comprised replacing the PG 9.5 antibody with rabbit immunoglobulin. Epidermal nerve counts were expressed as the number of fibers per millimeter of epidermis.

PARP activity was measured using immunohistochemical method that quantifies the accumulation of PAR, the product of the PARP activation (Obrosova et al., 2004;Szabó et al., 2002). PAR was assessed as we described previously (Tahrani *et al.*, 2012b). Paraffin sections (4µm) were dewaxed and endogenous peroxidases removed and blocked. After antigen retrieval, mouse monoclonal anti PAR antibody (1:400) (Enzo Life Sciences, Exeter, UK) was applied and then washed. Sections were incubated with ImPRESS universal reagent (Vector Laboratories). Immunoreactivity was revealed using diaminobenzidine (Vector Laboratories) and sections counterstained with haematoxylin. The number of stained and unstained endothelial nuclei were counted in ten fields from each section, using 400X magnification resulting in 300-400 nuclei being assessed. The results are expressed as % PAR stained nuclei (Szabó *et al.*, 2002).

Foot ulceration was assessed in the whole of the original study population (i.e. not only those who consented for skin biopsies) during foot exam, and based on question 8 of the MNSIq “Have you ever had an open sore on your foot?”

All Laboratory, sleep and clinical assessments were blinded.

Data analysis was performed using SPSS 22.0 software (SPSS Inc, Chicago, USA). Data are presented as mean (SD). Differences between independent groups were assessed by analysis of variance (ANOVA). Categorical variables were compared using the Chi-squared test or Fisher’s exact test. Correlations between continuous variables were performed using the Pearson or Spearman tests. Multiple linear regression (forced entry method) was used to assess independent associations of continuous outcomes and independent variables (for example the relationship between IENFD and OSA and AHI). Non-normally distributed variables were log transformed when used in the linear regression models (AHI was transformed to log (AHI+1), 1 was added as some AHI values were 0). Dummy variables were used in linear regression models if the independent variable was categorical with more than 2 categories (such as OSA status: no OSA, mild OSA and moderate to severe OSA). In multiple linear regression, residual distribution was examined and multicollinearity was assessed using correlations, the tolerance, variance inflation factor (VIF) and condition index. To assess

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whether OSA status or OSA severity are associated with DFU, multiple logistic regression (forced entry method) was used where DFU was the dependent variable and OSA and other variables were the independent variables. Variables included in the regression models were based on known outcome-related risk factors. A p value < 0.05 was considered significant.

Results

Data regarding DFU was available in the whole population of the original study (n=234), of which 52 patients agreed to have skin biopsies (Figure 1). PAR staining was available for all 52 patients while IENFD results were available for 48 patients. Of the 52 patients who had skin biopsies, the prevalence of OSA was 83% (43/52), with 24 (46%) mild OSA and 19 (37%) moderate to severe OSA. Of the original study population the prevalence of no OSA, mild OSA and moderate-severe OSA was 36% (83/234), 39% (90/234) and 26% (61/234).

The skin biopsy study population characteristics in accordance with OSA severity are summarized in Table 1. There were no statistically significant difference between patients with and without OSA with regards to important characteristics including age, diabetes duration, lipids profile, HbA1c, blood pressure (BP) and body mass index (BMI). There was a non-significant stepwise reduction in estimated glomerular filtration rate (eGFR) between patients with no OSA and those with mild and moderate to severe OSA as reported previously (Tahrani et al., 2013a). Patients with OSA had higher MNSIe scores indicating more DPN in patients with OSA as previously reported (Tahrani *et al.*, 2012a). There were more men and more White Europeans in patients with OSA, but these differences were not statistically significant. There were no differences in alcohol intake or smoking between groups. Insulin prescription was similar across groups and a high proportion of all groups were prescribed BP lowering and lipid lowering treatments.

OSA and IENFD

Results of IENFD were available in 48 patients (4 patients did not have enough tissue left to perform PAR immunostaining but not IENFD) , 9 patients with no OSA, 22 with mild OSA and 17 with moderate to severe OSA.

In ANOVA, there was a significant reduction in IENFD across groups (12.75±1.93 vs. 10.55±1.62 vs. 9.42±1.16 fibers/mm of epidermis for no OSA, mild OSA and moderate to severe OSA respectively, p<0.001) (Figure 2). IENFD correlated negatively with the AHI (r=-0.51, p<0.001) and ODI (r=-0.33, p=0.02). There were no associations between IENFD and time spent with oxygen saturation < 90% (r=0.08, p=0.57) or nadir nocturnal oxygen saturation (r=0.02, p=0.9).

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Using multiple linear regression, where IENFD was the dependent variable and OSA, age, gender, ethnicity, diabetes duration, HbA1c and BMI were the independent variables (R^2=0.43); mild OSA (B=-2.19, p=0.002) and moderate to severe OSA (B=-3.45, p<0.001) were associated with IENFD. Replacing OSA with log (AHI+1) in the model showed that IENFD was associated with log (AHI+1) (R^2=0.37) (B=-2.45, p<0.001). The regression coefficient suggests that lower AHI values were associated with higher IENFD. Similar results were found when log (ODI+1) was used in the model instead of log (AHI+1) (R^2=0.29) (B=-2.10, p=0.004).

OSA and PAR

There was a statistically non-significant trend of increasing %PAR stained nuclei with worsening OSA (55.17±20.91 vs. 58.89±17.24 vs. 66.84±15.73 for patients with no OSA, mild OSA and moderate to severe OSA respectively, p=0.18) (Figure 3). Log (AHI+1) correlated with %PAR stained nuclei (r=0.29, p=0.039) but there was no significant correlation between log (ODI+1) and %PAR stained nuclei (r=0.23, p=0.097).

Using the same multiple linear regression model described above but replacing IENFD with %PAR stained nuclei as the dependent variable (R^2=0.20) showed no relationship between %PAR stained nuclei and mild OSA (B=5.86, p=0.4,) or moderate to severe OSA (B=14.33, p=0.068). However, there was an association between log (AHI+1) and %PAR stained nuclei when log (AHI+1) was used in the model instead of OSA (B=13.67, p=0.025). The regression coefficient suggests that higher AHI was associated with higher %PAR stained nuclei.

OSA and DFU

The baseline characteristics of the original study (n=234) in relation to OSA status are summarized in Table 2 and were published previously(Tahrani *et al.*, 2012a). Patients with OSA were older, heavier and had longer diabetes duration and higher prevalence of peripheral vascular disease (PVD, based on the patients electronic records). OSA was also more common in men and in White Europeans. OSA patients were prescribed more insulin and anti-hypertensive medication compared to those without OSA.

The prevalence of DFU on clinical examination was 3.4% (n=8). There was a stepwise increase in the prevalence of DFU in patients with no OSA vs. mild OSA vs. moderate to severe OSA (0% (0) vs. 2.2% (2) vs. 10% (6), p=0.004).Due to the small number of patients with DFU on clinical examination, we could not perform logistic regression to assess independent associations.

Based on question 8 of the MNSIq, 47/234 (20.1%) patients had past history of DFU. The prevalence of a past history of DFU was significantly greater in patients with OSA (7.1% (6/84) vs. 28.1% (25/89)

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vs. 26.2% (16/61) for patients with no OSA, mild OSA and moderate to severe OSA respectively, X^2=13.75, p=0.001).

Using the presence of DFU past history (based on the MNSIq) as the dependent variable and OSA, age, gender, ethnicity, diabetes duration, Presence of PVD, BMI, HbA1c, and insulin treatment (Nagelkerke R^2= 0.25) showed that OSA was associated with past history of DFU (OR 3.34, 95%CI 1.19-9.38, p=0.022). Using the same model but replacing OSA (binary yes/no category) with OSA severity showed that mild OSA was associated with past history of DFU (OR 3.69, 95%CI 1.28-10.63, p=0.016) and there was a statistically non-significant association with moderate to severe OSA (OR 2.88, 95%CI 0.87-9.54, p=0.084).

Conclusions

We have shown that OSA and AHI are associated with lower IENFD, AHI is associated with PARP activation and OSA is associated with DFU in patients with Type 2 diabetes. To the best of our knowledge, this is the first report to describe the relationships between OSA and IENFD, PARP activation and DFU in patients with Type 2 diabetes.

IENFD has been shown to have high sensitivity and specificity to diagnose DPN, particularly small fiber neuropathy (England *et al.*, 2009;Lauria *et al.*, 2010;Malik *et al.*, 2011). Intra- and inter-observer variability for the assessment of IENFD demonstrates good agreement (Malik *et al.*, 2011). IENFD was found to correlate inversely with thermal thresholds and pain severity(Malik *et al.*, 2011). The lower IENFD in patients with OSA and Type 2 diabetes vs. patients with Type 2 diabetes only suggest that OSA is associated with small fiber neuropathy in patients with Type 2 diabetes which complements our previous finding that OSA was associated with clinically evident DPN and foot insensitivity suggesting that OSA in patients with Type 2 diabetes is associated with both small and large fibers neuropathy. Even mild OSA was independently associated with lower IENFD, which might reflect the relatively long diabetes duration in these patients that could amplify the impact of mild OSA in vulnerable tissues. Thus, assessing patients with shorter diabetes duration might yield different results. This finding was also similar to our previous study showing that mild OSA was independently associated with clinically evident DPN(Tahrani *et al.*, 2012a). Obesity also is an established risk factor for DPN and SDB (Tahrani *et al.*, 2013b;Tesfaye et al., 2005); in addition IENFD has been shown to correlate negatively with BMI (Smith and Singleton, 2013). However, in this study there were no significant differences in BMI between groups and the associations between IENFD and SDB remained significant despite adjustment for BMI in the multivariable analysis.

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PARP activation, secondary to oxidative stress-induced DNA damage, has been shown to play an important role in the pathogenesis of DPN and endothelial dysfunction in patients with diabetes (Obrosova *et al.*, 2004;Szabó *et al.*, 2002). In our study, PARP activation was independently associated with higher AHI; this is likely to be due to DNA damage secondary to oxidative stress in patients with OSA and type 2 diabetes. We have shown previously that OSA in patients with type 2 diabetes was associated with increased oxidative and nitrosative stress (Tahrani *et al.*, 2012a). In addition, PARP activation offers another possible mechanism to explain the cross-sectional and longitudinal associations observed between OSA and microvascular complications and endothelial dysfunction observed in patients with type 2 diabetes (Altaf et al., 2015;Tahrani *et al.*, 2012a;Tahrani *et al.*, 2013a;Tahrani *et al.*, 2013b). PARP inhibition has been shown to alleviate oxidative stress and reverse DPN and neuropathy in diabetic rodents (Drel et al., 2010;Drel et al., 2011;Lupachyk et al., 2011). Intermittent hypoxia has been shown to result in oxidative stress and PARP activation in in-vitro non-diabetic rodents (Chiu et al., 2012).

We also found that OSA is independently associated with DFU in patients with Type 2 diabetes. This association was independent of important risk factors such as PVD, BMI, age, diabetes duration and HbA1c. Considering that OSA is associated with DPN, endothelial dysfunction, oxidative stress, nitrosative stress, lower IENFD and PARP activation, this association between OSA and DFU may not be surprising. A recent in-vitro study showed that the serum from patients with OSA and the metabolic syndrome resulted in impaired endothelial wound healing compared to healthy controls (Briançon-Marjollet et al., 2014). However, a small study of 25 patients with OSA showed no evidence of impaired wound healing following partial foot amputation (Andrews et al.,). A recently published case series (n=3) suggested that CPAP treatment aided DFU healing in 2 patients with undiagnosed OSA and DFU (Vas et al., 2015). The impact of OSA on DFU and wound healing in patients with type 2 diabetes requires further prospective and interventional studies.

This study has several limitations, most importantly its cross-sectional design where causality cannot be proven. Although several reasons might explain how OSA might result in lower IENFD, PARP activation and DFU, it is plausible for neuropathy to result or worsen OSA by its impact on upper airway function and the ventilatory drive. Hence, it is possible that the relationship could be bi-directional. The rather small sample size is another limiting factor but not many patients were willing to have skin biopsy which is a mini-invasive procedure, but 52 patients is comparable (and even more) than many other studies of skin biopsies in patients with Type 2 diabetes.

Our study also has strengths. The population of the study were well characterized and this is the first report to examine the relationship between OSA and IENFD, PARP and DFU in patients with type 2

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diabetes. This is also the first report to show a relationship between OSA and IENFD and PARP activation in humans.

In summary, OSA was associated with lower IENFD, PARP activation and DFU in patients with Type 2 diabetes. PARP activation could be one of the mechanisms relating OSA to microvascular dysfunction and complications in patients with Type 2 diabetes. Whether PARP inhibition would be beneficial requires further studies. The impact of OSA on IENFD, PARP and DFU requires further prospective studies. Whether continuous positive airway pressure treatment can improve IENFD, inhibit PARP and/or reduce the incidence/improve the healing of DFU requires interventional studies.

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Authors contributions:

QAA: researched data, wrote and reviewed manuscript

AA: researched data, reviewed the manuscript

MKP: researched data, reviewed the manuscript

NTR: Reviewed paper and reviewed statistical analysis

AAT: hypothesis, obtained funding, researched data, laboratory work, wrote and reviewed manuscript

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Table 1: The characteristics of patients who had skin biopsies in relation to OSA status. BP: blood pressure; BMI: body mass index; PVD: peripheral vascular disease; eGFR: estimated glomerular filtration rate, EDS: excessive daytime sleepiness (i.e. Epworth sleepiness score ≥ 11).

	No OSA (n = 9)	Mild OSA (n = 24)	Moderate to severe OSA (n = 19)	p
Age, yr	59.57 (8.86)	59.66 (12.49)	62.62 (8.42)	0.385
Male	4 (44.4%)	19 (79.2%)	25 (78.9%)	0.104
White	6 (66.7%)	18 (75%)	17 (89.5%)	0.317
Diabetes duration, yr	12 (8 – 14)	13 (10 – 18.5)	15 (7 – 23)	0.437
Hba1c, %	7.98 (1.65)	8.5 (1.26)	8.01 (1.29)	0.618
Total cholesterol, mmol/L	3.77 (0.73)	3.79 (0.79)	3.89 (1.15)	0.25
HDL, mmol/L	1.23 (0.35)	1.13 (0.38)	1.16 (0.33)	0.995
LDL, mmol/L	1.74 (0.83)	1.86 (0.72)	1.77 (0.90)	0.673
Triglycerides, mmol/L	1.71 (0.95)	1.74 (0.86)	1.92 (0.86)	0.878
Systolic BP, mmHg	130.2 (18.17)	135.5 (14.33)	132.18 (23.02)	0.328
Diastolic BP, mmHg	81.66 (8.34)	79.77 (10.61)	76.1 (7.30)	0.667
Epworth Sleepiness Score	10.67 (4.72)	8.13 (4.87)	10.72 (4.50)	0.160
EDS	5 (55.6%)	7 (29.2%)	10 (52.6%)	0.204
BMI, kg/m^2	35.61 (12.89)	34.41 (6.01)	37.6 (8.76)	0.145
Waist/hip ratio	0.96 (0.08)	1.00 (0.04)	1.01 (0.06)	0.146
MNSIe score	0.5 (0 – 1.5)	3 (2 – 4.75)	4 (3.5 – 5)	<0.05
e-GFR, $ml/min/1.73m^2$	91.75 (28.89)	82.41 (29.29)	74.68 (26.01)	0.974
Alcohol	5 (55.6%)	15 (62.5%)	9 (47.4%)	0.611
Smoking (current or ex)	5 (55.6%)	11 (45.8%)	8 (42.1%)	0.800
Insulin	6 (66.7%)	14 (58.3%)	11 (57.9%)	0.893
GLP-1	1 (11.1%)	1 (4.2%)	2 (10.5%)	0.676
Oral antidiabetic treatment	8 (88.9%)	24 (100%)	16 (84.2%)	0.142
Antihypertensive agents	7 (77.8%)	20 (83.3%)	19 (100%)	0.129

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Lipid lowering treatment	9 (100%)	20 (83.3%)	19 (100%)	0.080
PVD	0 (0.0%)	1 (4.2%)	3 (15.8%)	0.232

Table 2: Participant characteristics in relation to SDB status. Data presented as median (IQR) or mean (SD). Categorical variables presented as number (% of SDB status).GFR: Glomerular Filtration Rate, TIA: Transient Ischaemic Attack, PVD: Peripheral Vascular Disease, EDS: Excessive Daytime Sleepiess. Analysis performed using the Chi-square test for categorical variables, the independent t test for normally distributed variables and the Mann-Whitney U test for non-normally distributed variables. Adapted with permission from (2).

	OSA- (n=83)	OSA+ (n=151)	P value
Male	34 (41%)	101 (67%)	< 0.001
Caucasians	32 (39%)	97 (64%)	<0.001
Age (years)	54.7 (11.9)	58.5 (11.3)	0.02
Diabetes Duration (years)	9 (5-15)	11 (7-17)	0.02
Body Mass Index (kg/m^2)	30.2 (27.3-35.0)	34.4 (30.9-39.5)	< 0.001
Waist circumference (cm)	105.5 (96.0-115.0)	116.0 (107.5-125.5)	< 0.001
Hip (cm)	106.0 (98.0-117.0)	114.0 (105.0-125.0)	< 0.001
Waist hip ratio	0.97 (0.93-1.02)	1.01 (0.96-1.05)	0.002
Neck circumference (cm)	38.0 (36.5-41.3)	43.0 (39.0-46.0)	< 0.001
Height (cm)	163.5 (8.3)	167.8 (10.0)	0.001
Systolic blood pressure (mmHg)	125.5 (115.0-135.5.0)	130.0 (123.5-140.0)	0.002
Diastolic blood pressure (mmHg)	78.50 (71.0-85.00)	78.00 (71.00-84.50)	0.88
HbA1c (%)	7.7 (7.0-8.7)	8.3 (7.3-9.3)	0.05
Total cholesterol (mmol/L)	3.7 (3.4-4.5)	3.7 (3.3-4.3)	0.57
Triglycerides (mmol/L)	1.5 (1.0-2.1)	1.8 (1.3-2.5)	0.03
HDL (mmol/L)	1.2 (0.9-1.4)	1.1 (0.9-1.2)	0.02
Estimated GFR (ml/min/1.73 m^2)	92.92 (25.16)	82.41 (26.41)	0.003

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TSH	1.6 (1.0-2.2)	1.7 (1.2-2.4)	0.32
Epworth sleepiness score	5.0 (2.0-12.0)	8.0 (4.0-13.0)	0.003
EDS	30.1% (n=25)	39.1% (n=59)	0.172
Smoking (current or ex-smoker)	32 (39%)	62 (41%)	0.71
Alcohol (drinks alcohol)	12 (15%)	12 (35%)	0.001
Oral anti-diabetes treatment	81 (98%)	137 (91%)	0.05
Insulin	34 (41%)	91 (60%)	0.005
Insulin dose (units)	61 (35-88)	80 (56-118)	0.007
ACE inhibitors	40 (48%)	69 (46%)	0.71
Anti-hypertensive agents	61 (74%)	129 (85%)	0.03
Lipid lowering treatment	71 (86%)	125 (83%)	0.58
Stroke or TIA	60 (7%)	18 (12%)	0.28
Ischemic heart disease	14 (17%)	33 (22%)	0.40
PVD	1 (1%)	10 (7%)	0.06
Albuminuria	20 (24%)	65 (43%)	0.007
Sight threatening retinopathy	17 (21%)	72 (48%)	< 0.001

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Figure 1: The study diagram

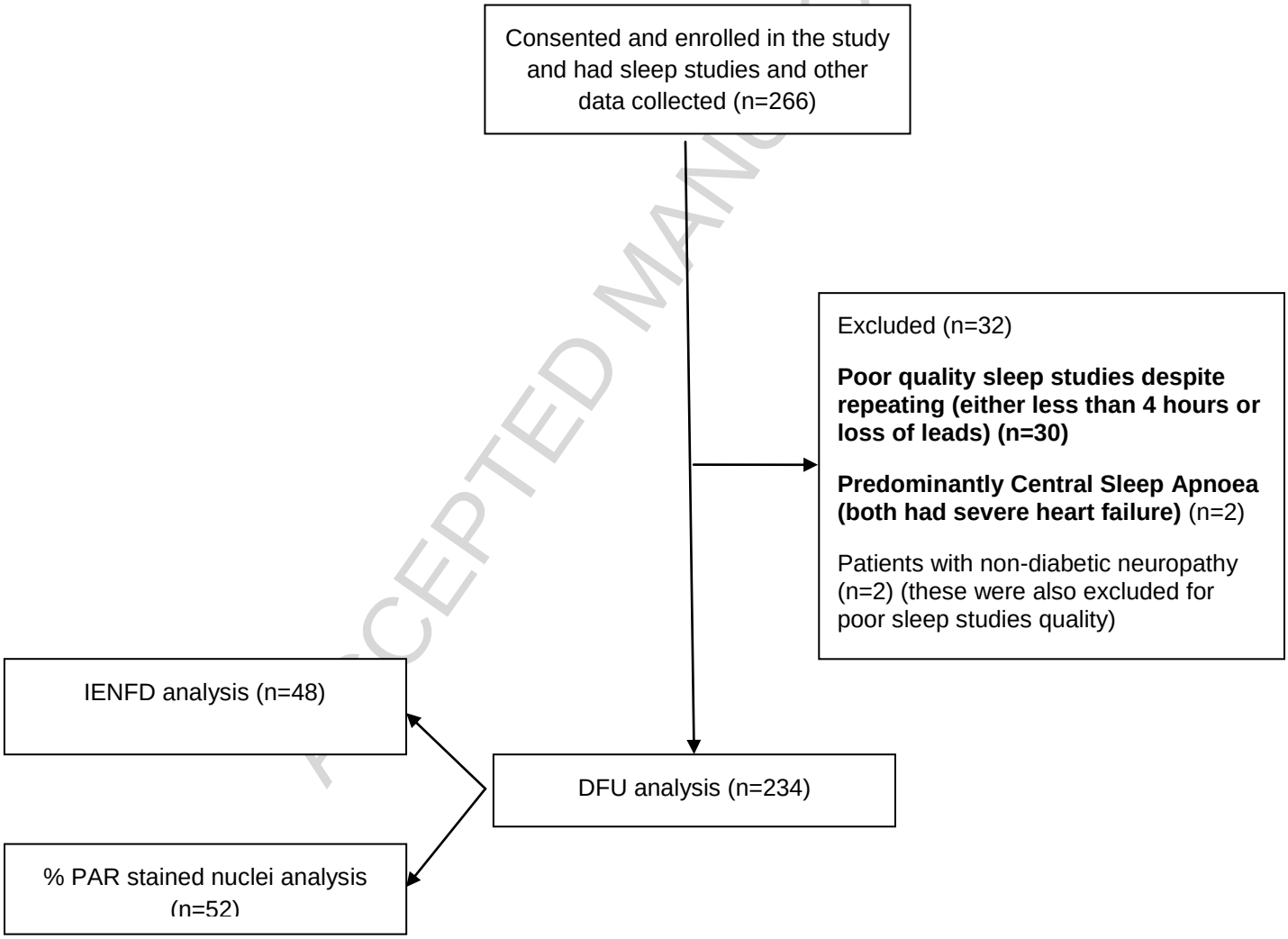

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Figure 2 - The relationship between obstructive sleep apnoea and Intra epidermal nerve fibre density (IENFD).

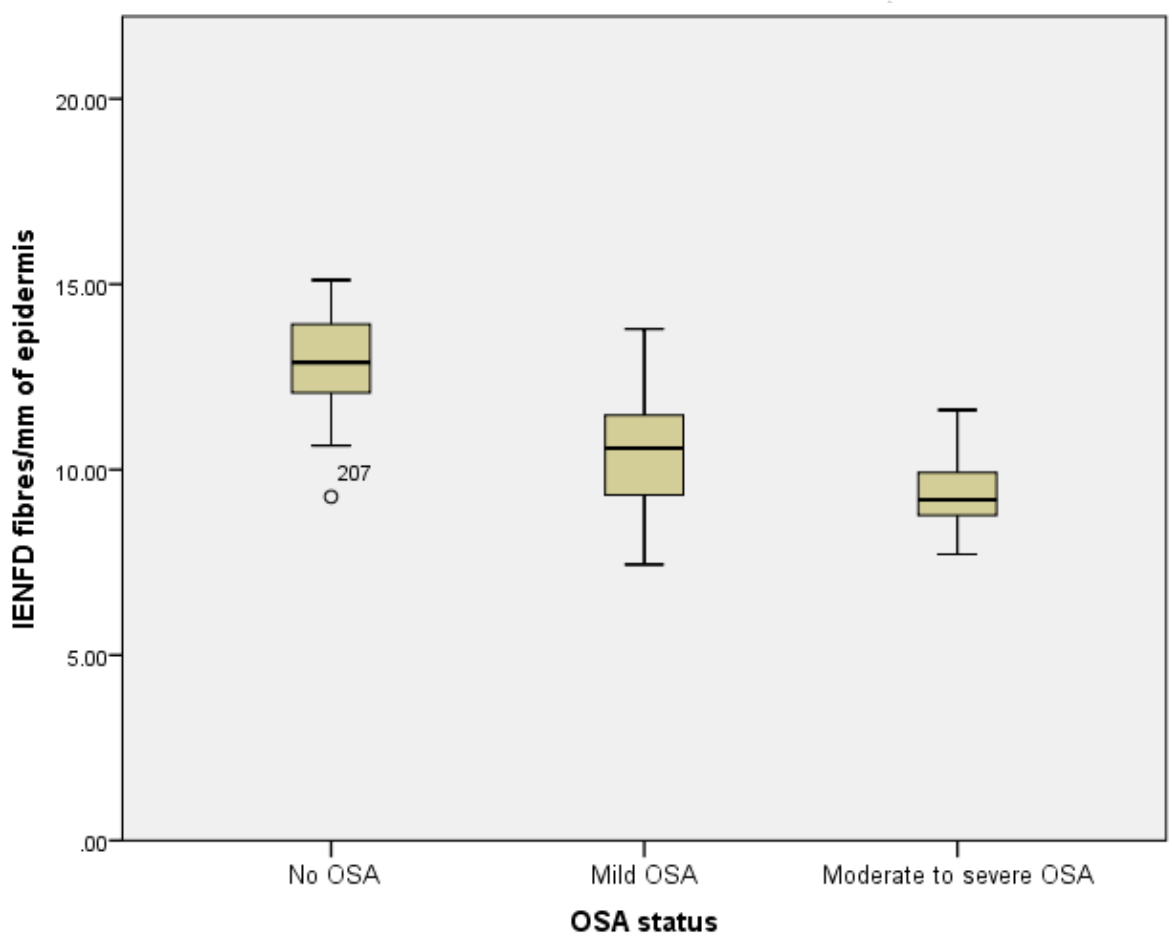

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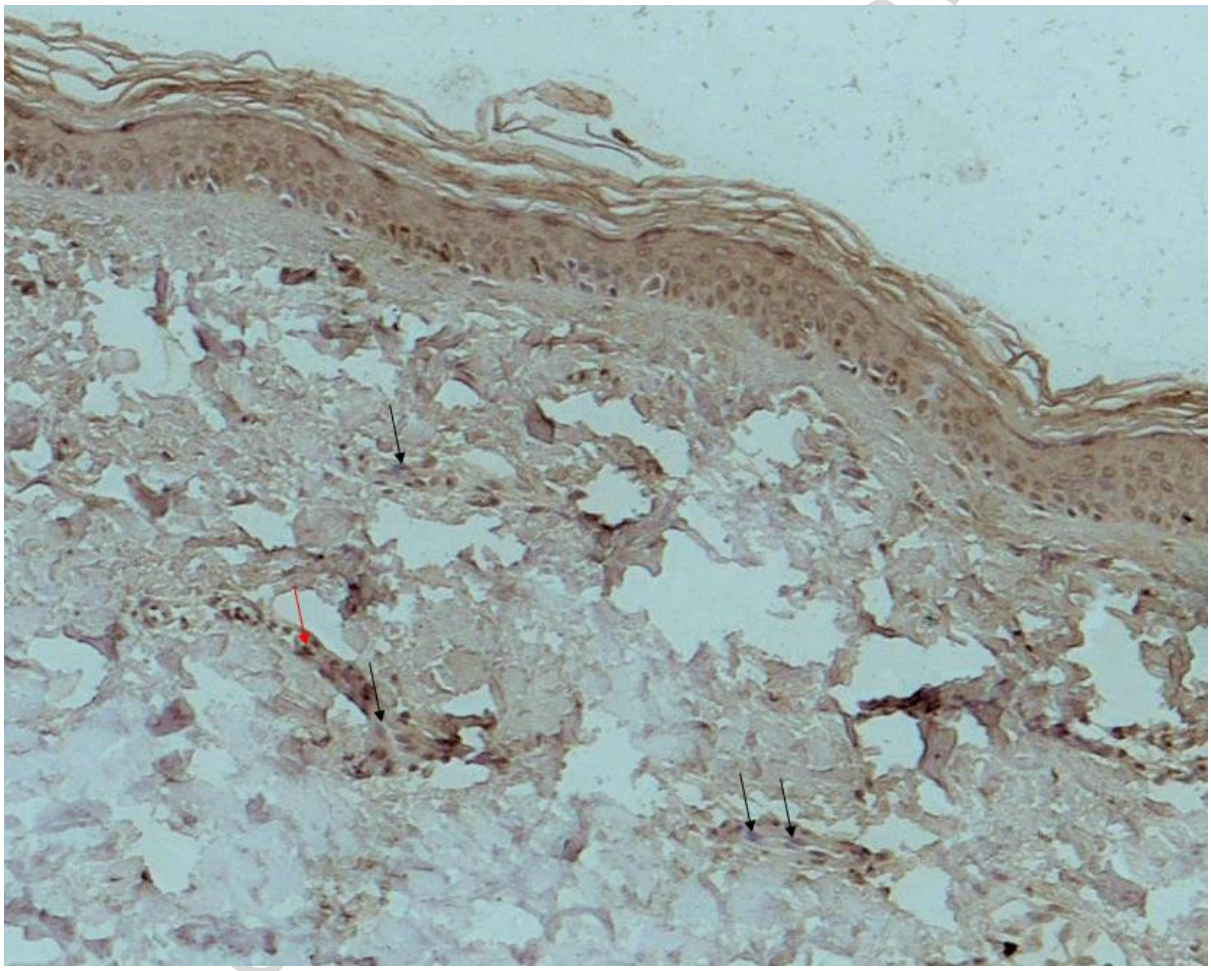
Figure 3: PAR immunostaining from A) patient with AHI of 1.6, B) patient with AHI 31.2. Black arrow: non PAR stained nuclei (purple), Red arrow: PAR stained nuclei (brown)

A:



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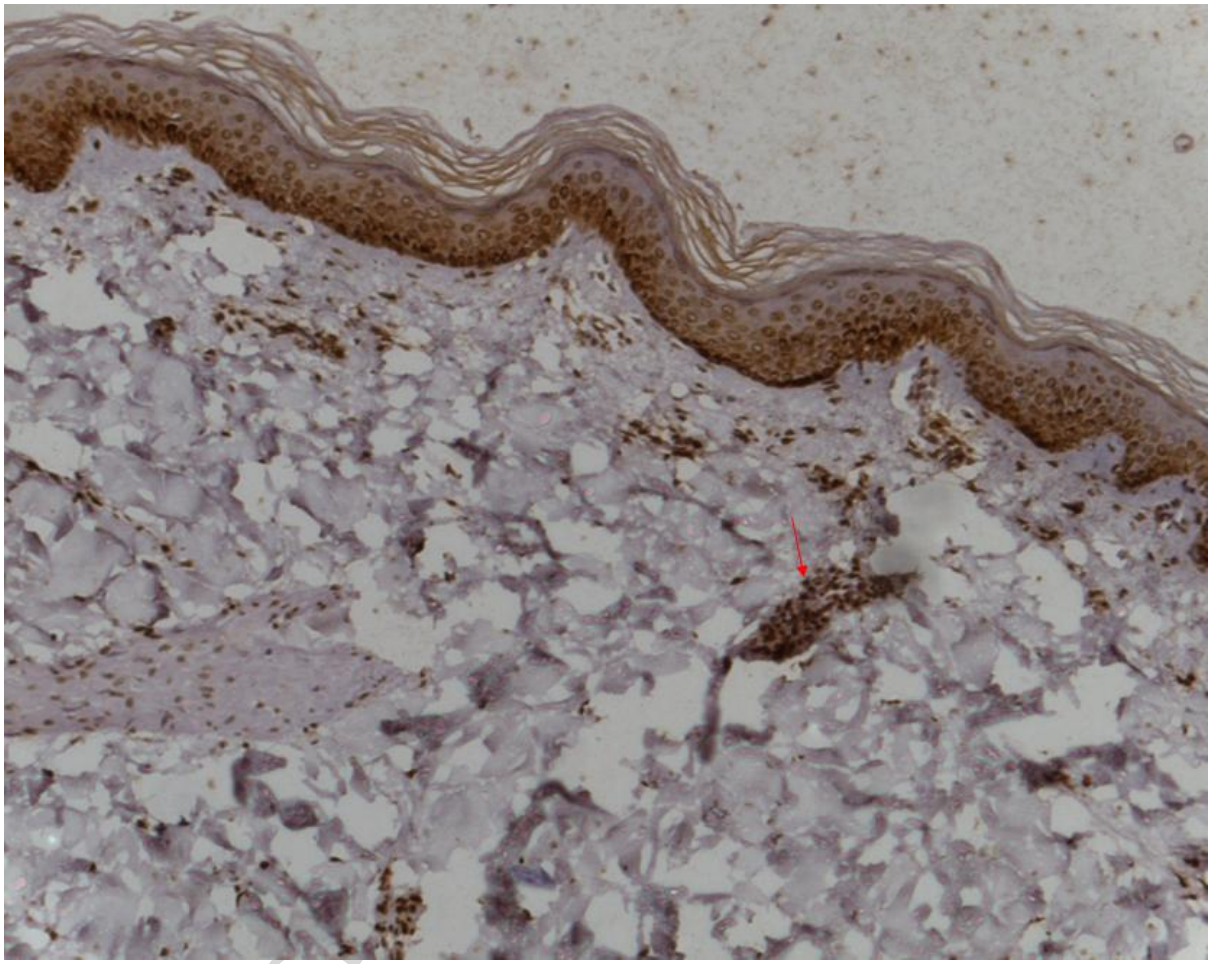

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Highlights

1. OSA is associated with lower IENFD in patients with Type 2 diabetes (T2D)
2. OSA is associated with small as well as large fibre dysfunction in patients with T2D.
3. OSA is associated with PARP activation in patients with T2D
4. OSA is associated with foot ulceration in patients with T2D.
5. The impact of CPAP on IENFD and foot ulceration in T2D need to be examined.

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