A Meta-Analysis of Efficacy of Carbon Dioxide Inhalation as a Challenge Test in Panic Disorder

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Objective: The objective of this study is to perform a systematic review and meta-analysis on whether patients with panic disorder (PD) and their healthy first-degree relatives have an increased sensitivity to carbon dioxide inhalation test compared to healthy controls (HC) or patients with psychiatric disorders other than panic disorder.

Method: The databases of PubMed, EMBASE and PsycNET were searched using Boolean operators "panic AND carbon dioxide" and "panic AND CO_2". Selected research articles were classified according to the carbon dioxide concentrations used in testing and the clinical characteristics of the samples. The assumption of heterogeneity across the studies was assessed by chi square based Q and I^2 statistics. Publication biases were explored by Begg-Mazumdar's and Egger's tests in addition to funnel graphics. Odds ratios representing effect size of the carbon dioxide inhalation procedure were calculated according to fixed effect and random effect models after obtaining percent weight effects of each study.

Results: Meta-Analysis was conducted on 33 research studies that include 2114 participants totally. Participants with PD experienced significantly more frequent panic attacks (PA) compared to HC following in both 5% (OR=14.713, 95% CI 7.532 – 28.739) and 35% carbon dioxide inhalation (OR=11.507, 95% CI 7.775 – 17.031). HC who have a first-degree relative with PD experienced PA approximately 3 times more than HC who have not a first-degree relative with PD (OR=2.658, 95% CI 1.678 – 4.212) following carbon dioxide inhalation test. Participants with PD experienced significantly more frequent PA than the patients with other psychiatric disorders following the carbon dioxide inhalation test (OR = 3.524, 95% CI 1.945 - 6.384).

Conclusion: There is an increased sensitivity of carbon dioxide inhalation in patients with PD and their healthy first-degree relatives. The role and possible mechanisms of carbon dioxide in etiology and physiopathology of PD should be studied extensively.

Keywords: Panic disorder, carbon dioxide, meta-analysis, first-degree relatives of PD patients, provocation

INTRODUCTION

Panic disorder (PD) is a mental disorder with a lifetime prevalence of 3.5-10.3% and the disabilities attributed to PD is found to be similar to those observed in major depressive disorder and certain neurotic disorders (Bener et al. 2015, Carta et al. 2015, Markowitz et al. 1989). PD has been the focus of various biological theories and biological research. The most widely used methods in the investigation of PD biology are the challenge tests. The biological and psychological measurements carried out during panic attacks (PA) induced by provocation tests help us understand the underlying processes of this disorder. Provocation tests include methods such as sodium lactate infusion, caffeine, yohimbine and tryptophan depletion, and flumazenil, cholecystokinin, and carbon dioxide inhalation (Bourin et al. 1998, Forsythe and Karekla 2002). The carbon dioxide inhalation test is a widely used method in studies as it is safe (Harrington et al. 1996, Prenoveau et al. 2006). However, individuals with PD find this test very disturbing (Feinstein et al. 2013). Most of the studies have shown that carbon dioxide sensitivity is elevated in subjects with PD compared to healthy controls (HC) and those with other psychiatric disorders. This means that having

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inhaled a certain proportion of carbon dioxide combined with oxygen, individuals with PD experience significantly more frequent PA than HC. Even healthy individuals with a first-degree family history of PD experience significantly more frequent PA than healthy individuals who do not have relatives with PD when inhaling carbon dioxide (Amaral et al. 2014). These findings suggest that there is an increased sensitivity to carbon dioxide inhalation in individuals with PD and that the tendency to such sensitivity is inherited by their healthy first-degree relatives even if PD symptoms are not manifest in them. However, vulnerability to anxiety, some comorbid psychiatric diagnoses such as smoking and phobias, menstrual period and conditions of the experimental setting may modify the frequency of PA occurrence after challenge tests (Abrahams et al. 2017, Blechert et al. 2013, Forsythe and Karekla 2002).

Carbon dioxide inhalation tests can be performed using differing concentrations of the gas and of administration methods. Majority of the studies reported in the literature have used carbon dioxide concentrations of 5% or 35%, while there are a few studies with concentrations of 5.5, 7 and 20%. The method generally involves inhalation of a single vital capacity, but some studies have also used normal respiration for a certain period of time by employing a mask or a cannula. Although it has been shown in some studies that the frequency of PA increases with increasing carbon dioxide concentration, the dose-response relationship has remained ambiguous (Gorman et al. 1994, Griez et al. 2007).

Despite the large number of studies employing the carbon dioxide inhalation test in individuals with PD, the reported frequencies of PA after the provocation test vary greatly between 0.15 and 0.90 (Blechert et al. 2010, Woods et al. 1986). PA frequency variation on such a wide scale not only causes uncertainty in planning new studies for estimating average risk, but also raises questions about the reliability of carbon dioxide inhalation test. Hence, the risk of having a PA when inhaling carbon dioxide by individuals diagnosed with PD and their asymptomatic healthy relatives, as compared to those diagnosed with psychiatric disorders other than panic disorder (OPD) and to HC is not definitely known.

The purpose of this meta-analysis is to determine whether or not there is an increase in the sensitivity to inhaling carbon dioxide in individuals with PD diagnosis as compared to patients with OPD as well as to healthy individuals with and without first degree relatives diagnosed with PD.

METHOD

Data Collection

The PubMed, EMBASE and PsycNETsearch engines were examined with the Boolean processors for "panic [Title/Abstract] AND (CO_2 [Title/Abstract] OR (carbon dioxide [Title/Abstract]". Only publications in English covering the period between 1985 and 2015 and involving subjects aged between 18 and 65 were included in the study. The flow and article selection chart of this meta-analysis is shown in Figure 1. After reviewing the abstracts of the listed articles, full texts of the selected articles were evaluated. Articles not reporting data on the frequency of PA after the carbon dioxide inhalation tests were excluded. If different concentrations of carbon dioxide were used in a given study, the data on the lowest concentration used were selected. Also, if the PD cases were compared to more than one type of mental disorder, data on the cases with highest PA frequency were included. A database file was prepared for the selected articles to record the first authorname(s), the year, and the data on the sample size, gender and mean age distribution, carbon dioxide concentrations used, the number of the people experiencing PA and the frequency PA following carbon dioxide inhalation tests.

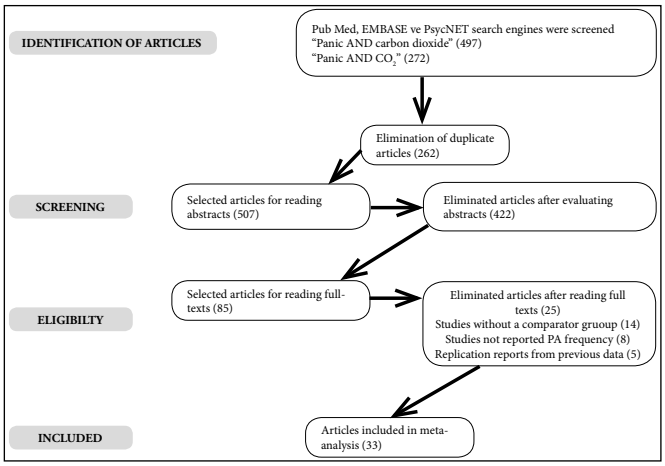

Figure 1. Selection Process of Articles Included in the Meta-Analysis.

Patients

Studies made with inpatients or outpatients were all included in the study. The PD comparison group included only those cases with PD diagnoses based on the DSM III, DSM-III-R or DSM-IV criteria. Studies that tested patients after psychotherapy, medical treatment or other procedures intended to treat their PD were excluded from the meta-analysis. The meta-analysis included only the pre-intervention (baseline) values of the studies investigating the effects of drugs on the sensitivity to carbon dioxide inhalation tests.

Statistics

Data from the included articles on PA incidence among subjects with PD diagnoses and the comparison groups

after a carbon dioxide inhalation test were entered in the database to enable the calculation of the odds ratios of the experimental group against the control group. A delta value of 0.5 was entered as a correction for the entries that had a value of 0. The studies were tested for homogeneity by means of the Cochrane's Q test and the I^2 value. Publication bias across the studies included in the meta-analysis were explored with funnel plots, and the Begg-Mazumdar's and Egger's tests. The weighted effect sizes of the studies were calculated for the fixed and random effect models. If homogeneity was secured, the odds ratios in the results of the fixed effect model were interpreted; and, if not, computations were based on the random effect model. All analyses were tested using the NCSS 11 (NCSS, LLC. Kaysville, Utah, USA, 2016) and the StatsDirect (StatsDirect Ltd. England, 2013) statistical softwares.

RESULTS

A pool of 2114 participants consisting of 949 with PD diagnosis, 602 HC, 372 with OPD diagnoses and 191 having a first degree relative with PD diagnosis was obtained from a total of 33 study articles. The sample consisted of 1212 females, 842 males and 60 subjects with unreported gender. The studies and the characteristics of the study groups in the studies are shown in Table 1. Selections were made from these articles by focus of analysis and the meta-analysis was completed. A large number of the studies had used carbon dioxide concentrations of 5% and 35% (Table

Table 1. General Features of the Studies Included in the Meta-Analysis

Studies	Compared Groups	Carbon Dioxide		Age (mean±sd)			Gender N (F/M)		
		Concentration (%)	Method	PD	HC	OPD	PD	HC	OPD
Alkın et al. 2007	PD and HC	35	1 vital capacity inhalation through face mask	34.3± 8.04	34.3±6.61	—	18/6	8/4	—
Antony et al. 1997	PD, Phobias and HC	5.5	15 mins breathing through face mask	32.47±7.76	30.87±7.70	49.07±10.61	11/4	7/8	11/4
Atlı et al. 2012	PD, SA and HC	35	1 or 2 vital capacity inhalations through face mask	30.2±9.9	29.2±9.2	31.0±10.0	26/12	24/16	25/6
Blechert et al. 2010	PD, SAD and HC	20	Discrete 1 vital capacity inhalations through oral tube	39.1±11.3	37.4±10.2	39.5±10.6	11/9	11/7	10/9
Bocola et al. 1998	PD and HC	7	Read's rebreathing method, 5 mins	NR	NR	—	NR	NR	—
Caldirola et al. 1997	PD and SAD	35	1 vital capacity inhalation through face mask	31.7±6.4	25.8±2.8	30.7±9.6	8/8	11/ 5	10/6
Coryell et al. 1997	HC having first-degree relative with PD and HC	35	1 vital capacity inhalation through face mask	—	26.2±3.8	22.1±3.9	—	7/8	7/4
Coryell et al. 2006	HC having first-degree relative with PD and HC	35	1 vital capacity inhalation through face mask	—	23.5±3.9	22.9±4.1	—	54/31	98/34
Fyer et al. 1987	PD and HC	35	2 vital capacity inhalations through face mask	36.0±6.8	29.40±1.7	—	NR	NR	—
Gorman et al. 1988	PD, Anxiety Disorders and HC	5	20 mins breathing through canopy	33.7±5.7	28.1±7.1	33.7±9.3	16/15	3/10	4/8
Gorman et al. 1990	PD, SAD and HC	35	1 vital capacity inhalation through face mask	34.7±8.2	27.1±5.6	34.9±8.5	16/10	7/7	8/14
Gorman et al. 1994	PD and HC	5 and 7	20 mins breathing	36.3±9.9	32.5±10.3	—	15/9	12/6	—
Gorman et al. 2004	PD and HC	5 and 7	20 mins breathing through canopy	32.96±6.92	30.46±7.04	—	7/18	1/12	—
Kent et al. 2001	PD, PMDD and HC	5 and 7	20 mins breathing through canopy	32.2±7.7	30.0±8.1	30.0±8.1	23/29	15/19	10/0

Table 1 Continue

Studies	Compared Groups	Carbon Dioxide		Age (mean±sd)			Gender N (F/M)		
		Concentration (%)	Method	PD	HC	OPD	PD	HC	OPD
Monkul et al. 2010	PD, HC having first-degree relative with PD and HC	35	1 vital capacity inhalation through face mask	33.9 ± 10.8	34.6±11.6	32.6±11.2	20/12	20/14	18/14
Niccolai et al. 2008	PD and HC	35	1 vital capacity inhalation through face mask	36.0±14.0	33.0±12.0	—	7/4	7/4	—
Papp et al. 1993	PD, SAD and HC	35	20 or 30 secs breathing through face mask	35.3±9.6	31.2±8.6	34.3±9.2	12/6	9/14	4/16
Perna et al. 1994.	PD and HC	35	1 vital capacity inhalation through face mask	30.9±8.9	25.7±3.8	—	41/30	26/18	—
Perna et al. 1995a	PD, HC having first-degree relative with PD and HC	35	1 vital capacity inhalation through face mask	33.1±11.1	28.4±8.0	41.0±12.2	48/36	24/20	9/14
Perna et al. 1995b	PD, OCD and HC	35	1 vital capacity inhalation through face mask	33.5±11.2	25.3±3.8	30.7±8.1	14/9	14/9	10/13
Perna et al. 1995c	PD, MDD and HC	35	1 vital capacity inhalation through face mask	32.1±10.3	25.2±4.1	49.2±12.0	12/8	11/9	17/2
Perna et al. 1995d	PD, HC and SPA	35	1 vital capacity inhalation through face mask	31.1±8.6	28.0±6.1	32.1±11.6	26/17	26/17	8/5
Perna et al. 1999	PD, GAD and HC	35	1 vital capacity inhalation through face mask	35.8±11.1	27.5±5.1	39.1±9.4	11/4	10/2	10/3
Perna et al. 2004	PD, ED and HC	35	1 vital capacity inhalation through face mask	NR	NR	NR	14/0	14/0	14/0
Rapee et al. 1992	PD, GAD, SAD, OCD and HC	5.5	15 mins breathing through face mask	32.9±8.9	31.4±9.4	34.4±9.8	50/25	14/11	53/40
Rassovsky et al. 2006	PD and HC	5	Read's rebreathing method, five mins or until CO2 reaches to 70 mm Hg	30.4±9.6	27.5±8.5	—	26/14	22/10	—
Sasaki et al. 1996	PD and HC	5	20 mins breathing through gas mask	39.1±2.6	28.3±2.6	—	NR	NR	—
Schutters et al. 2011	PD, SAD and HC	35	NR	32.8±9.5	30.2±10.2	32.8±10.0	10/6	10/6	10/6
van Beek et al. 2000	HC having first-degree relative with PD and HC	35	1 vital capacity inhalation through face mask	—	30.56±12.31	30.8±12.08	—	21/29	21/29
Verburg et al. 1995	PD, GAD and HC	35	1 vital capacity inhalation through face mask	37.9±9.5	—	41.0±9.1	7/2	6/3	—
Verburg et al. 1998	PD and MDD	35	1 vital capacity inhalation through face mask	36.5±7.8	—	34.9±8.0	13/10	—	10/2
Woods et al. 1986.	PD and HC	5	Read's rebreathing method, until end tidal max CO2 reaches to 70 mm Hg	37.9±2.5	39.3±2.4	—	12/2	9/1	—
Woznica et al. 2015	PD and BN	35	1 vital capacity inhalation through mouth piece	26.00 ± 7.92	24.13±5.95	25.14±7.67	15/0	30/0	14/0

BN: Bulimia nervosa, ED: Eating disorders, GAD: Generalized anxiety disorder, HC: Healthy controls, MDD: Major depressive disorder, NR: Not reported, OCD: obsessive compulsive disorder, OPD: Psychiatric diagnosis other than panic disorder, PD: Panic disorder, PMDD: Premenstrual dysphoric disorder, SA: Separation anxiety, SAD: Social anxiety disorder, SPA: Sporadic panic attacks.

Table 2. Meta-Analysis of Studies Comparing PA Frequency Between PD and HC Groups by 5% Carbon Dioxide Inhalation Test*

Study	In PD (PA+/Total N)	In HC (PA+/Total N)	Odds Ratio	95 % CI	Weight %	
					Fixed	Random
Antony et al. 1997	6/15	0/15	21.211	1.069 - 420.823	4.649	5.047
Blechert et al. 2010	3/20	0/18	7.400	0.356 - 153.845	6.851	4.892
Bocola et al. 1998	8/9	0/10	119.000	4.277 - 3311.296	1.118	4.072
Gorman et al. 1988	12/31	1/13	7.579	0.870 - 65.999	13.524	9.618
Gorman et al. 1994	6/21	0/17	14.677	0.763 - 282.288	6.068	5.153
Kent et al. 2001	26/50	3/34	11.194	3.025 - 41.428	26.844	26.31
Rapee et al. 1992	49/75	3/25	13.821	3.779 - 50.542	24.428	26.794
Rassovsky et al. 2006	13/40	0/32	31.909	1.813 - 561.680	5.819	5.477
Sasakı et al. 1996	5/13	0/15	20.059	0.985 - 408.388	4.437	4.961
Woods et al. 1986	9/10	4/10	13.500	1.197 - 152.218	6.264	7.676
Total (fixed effects) χ^2=86.23, df=1, p<0.001)	137/284	11/189	14.713	7.532 - 28.739	100	
Total (random effects) χ^2=60.213, df=1, p< 0.001)	137/284	11/189	14.257	6.328 - 22.371	100	
Test of heterogeneity			Q=2.635, df=9, p=0.977; I^2=0.00% (95% CI 0.00-97.7)			

*PD: Panic disorder, HC: Healthy controls, PA+: Number of participants experiencing panic attack following carbon dioxide challenge test, N: Number of total participants.

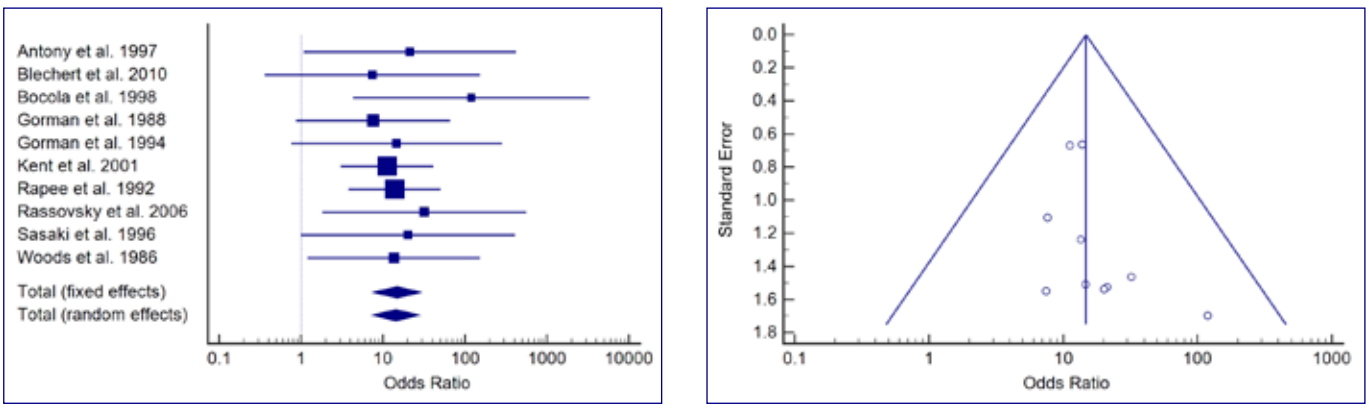

Figure 2. a) Forest Plot Graph of Odds Ratios From Studies Comparing PD and HC Groups by 5% Carbon Dioxide Inhalation Test, **B)** Funnel Plot for Publication Bias
Odds Ratio (fixed effects) = 14.713 (95%CI 7.532 - 28.739) in favor of PD

1). To reduce loss of data, a study using 20% carbon dioxide concentration was included in the 35% group. Also, a study using 7% concentration and two others using 5.5% concentrations were included in the 5% group to complete the meta-analysis in two groups as the 5% and the 35% carbon dioxide inhalation tests.

Comparison of the PD and HC Groups

When the studies were divided into the 5% and 35% subgroups, it was observed that after carbon dioxide inhalation in both carbon dioxide concentrations, the subjects diagnosed with PD experienced PA with significantly higher frequency as compared to the HC. The respective statistics are presented in Table 2 and the odds ratios and publication bias funnel plots are shown in Figures 2a and b. The frequency of PA after a 5% CO_2 inhalation was observed to be significantly higher in patients with PD than in the HC (Table 2 and Figure 2a). Significant heterogeneity was not seen in the included studies of the meta-analysis (I^2 = 0.00, p = 0.977) and data suggesting any significant publication bias were not found (Begg-Mazumdar's: Kendall's tau = 0.378, p = 0.156; Egger's test = 0.655, 95% CI = -.372 - 1.682, p = 0.179). After a 5% carbon dioxide inhalation test, the subjects with PD experienced PA nearly 15 times more than the HC. Also, analysis of the results of the 35% carbon dioxide inhalation tests revealed that the PD group experienced significantly more frequent PA than the HC group following the provocation test (Table 3 and Figure 3a). Significant heterogeneity was not seen in the studies included in the meta-analysis for 35% carbon dioxide inhalation test (I^2 = 21.73, p = 0.196) and data suggesting significant publication bias were not obtained (Figure 3b) (Begg-Mazumdar's: Kendall's tau = 0.072, p = 0.709; Egger's

Table 3. Meta-Analysis of Studies Comparing PA Frequency Between PD and HC Groups by 35% Carbon Dioxide Inhalation Test*

Study	In PD (PA+/Total N)	In HC (PA+/Total N)	Odds Ratio	95 % CI	Weight %	
					Fixed	Random
Alkın et al. 2007	10/24	2/12	3.571	0.639 - 19.974	8.414	6.119
Atlı et al. 2012	21/38	7/40	5.824	2.066 - 16.417	16.504	12.057
Caldirola et al. 1997	11/16	1/16	33.000	3.363 - 323.825	1.69	3.856
Fyer et al. 1987	5/8	0/5	17.286	0.712 - 419.951	1.262	2.124
Gorman et al. 1990	13/26	3/14	3.667	0.826 - 16.273	10.547	7.588
Gorman et al. 2004	11/23	2/13	5.042	0.908 - 28.000	7.212	6.158
Monkul et al. 2010	15/32	8/34	2.868	1.000 - 8.224	22.291	11.838
Niccolai et al. 2008	6/11	1/11	12.000	1.118 - 128.842	2.459	3.608
Papp et al. 1993	13/18	1/23	57.200	6.005 - 544.817	1.319	3.943
Perna et al. 1994	34/71	2/44	19.297	4.336 - 85.882	6.961	7.568
Perna et al. 1995a	43/84	1/44	45.098	5.933 - 342.791	3.465	4.707
Perna et al. 1995b	12/23	0/23	51.087	2.773 - 941.012	1.296	2.508
Perna et al. 1995c	11/20	1/19	22.000	2.443 - 198.149	2.496	4.116
Perna et al. 1995d	20/43	1/43	36.522	4.600 - 289.937	2.893	4.545
Perna et al. 1999	12/15	3/12	12.000	1.947 - 73.974	3.606	5.615
Perna et al. 2004	11/14	2/14	22.000	3.076 - 157.347	2.318	4.949
Schutters et al. 2011	12/16	3/16	13.000	2.398 - 70.463	4.057	6.294
Woznica et al. 2015	5/15	0/30	31.952	1.625 - 628.344	1.208	2.409
Total (fixed effects) (χ^2=192.041, df=1, p<0.001)	265/497	38/413	11.507	7.775 - 17.031	100	
Total (random effects) (χ^2=93.308, df=1, p<0.001)	265/497	38/413	10.890	6.708 - 17.679	100	
Test of heterogeneity	Q=21.719, df=17, p=0.196; I^2=21.73% (95% CI 0.00-66.10)					

*PD: Panic disorder, HC: Healthy controls, PA+: Number of participants experiencing panic attack following carbon dioxide challenge test, N: Number of total participants.

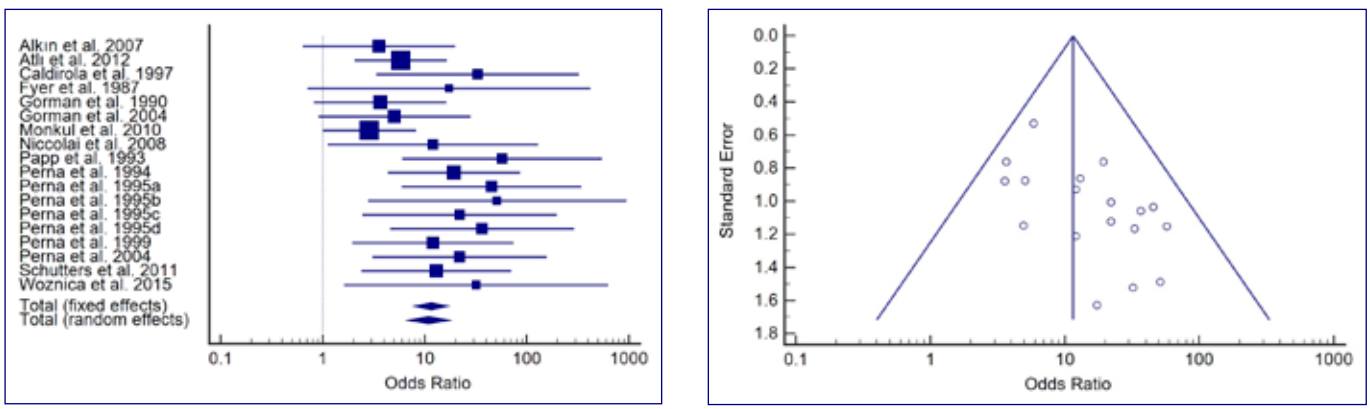

Figure 3. a) Forest Plot Graph of Odds Ratios From Studies Comparing PD and HC Groups by 35% Carbon Dioxide Inhalation Test, **B)** Funnel Plot for Publication Bias

Odds Ratio (fixed effects) = 11.507 (95%CI 7.775 - 17.031) in favor of PD

test = 0.748, 95% CI = -0.979 – 2.476, p = 0.372). The subjects with PD experienced PA nearly 12 times more than healthy controls after a 35% carbon dioxide inhalation test.

Comparison of the Subjects with PD and Those with Other Psychiatric Disorders (OPD)

The subjects with PD experienced significantly more PA than those with OPD after a carbon dioxide inhalation test (Table 4 and Figure 4a). However, considerable heterogeneity was observed across the studies included in the meta-analysis (I^2 = 60.07%, p<0.001). The funnel plots (Figure 4b) and bias tests of the studies supported the absence of publication bias (Begg-Mazumdar's: Kendall's tau = 0.281, p = 0.112; Egger's test = 1.11, 95% CI = -0.724 – 2.944, p = 0.218). Considering the foregoing, it can be said that the odds ratio of the individuals with PD to experience more PA than those with OPD after a

Table 4. Meta-Analysis of Studies Comparing PA Frequency Between PD and OPD Groups by Carbon Dioxide Inhalation Test*

Study	In PD (PA+/Total N)	In OPD (PA+/Total N)	Odds Ratio	95 % CI	Weight %	
					Fixed	Random
Antony et al.1997	6/15	3/15	2.667	0.521 - 13.656	3.668	5.824
Atlı et al. 2012	21/38	16/31	1.158	0.447 - 2.999	16.065	8.367
Blechert et al. 2010	3/20	0/19	7.8	0.376 - 161.876	0.87	2.471
Caldirola et al. 1997	11/16	9/16	1.711	0.403 - 7.271	5.731	6.401
Gorman et al. 1988	12/31	0/12	16.026	0.869 - 295.534	0.883	2.627
Gorman et al. 1990	13/26	8/22	1.75	0.549 - 5.582	8.83	7.492
Kent et al. 2001	26/50	5/10	1.083	0.279 - 4.213	8.15	6.774
Papp et al. 1993	13/18	6/20	6.067	1.486 - 24.764	3.217	6.560
Perna et al. 1995b	12/23	1/23	24	2.755 - 209.071	0.975	4.877
Perna et al. 1995c	11/20	1/20	23.222	2.585 - 208.623	0.917	4.962
Perna et al. 1995d	20/43	7/13	0.745	0.215 - 2.587	11.716	7.182
Perna et al. 1999	12/15	1/13	48	4.352 - 529.377	0.437	4.239
Perna et al. 2004	11/14	1/14	47.667	4.318 - 526.191	0.437	9.648
Rapee et al. 1992	49/75	28/93	4.375	2.284 - 8.380	17.659	4.236
Schutters et al. 2011	12/16	8/16	3	0.671 - 13.404	4.075	6.243
Verburg et al. 1995	8/9	2/9	28	2.067 - 379.265	0.453	3.803
Verburg ve ark 1998	15/23	12/12	0.073	0.004 - 1.391	11.703	2.581
Woznica et al. 2015	5/15	3/14	1.833	0.346 - 9.720	4.216	5.715
Total (fixed effects) (χ^2= 56.597, df=1, p<0.001)	260/467	111/372	3.089	2.284 - 4.177	100	
Total (random effects) (χ^2=17.254, df=1, p<0.001)	260/467	111/372	3.524	1.945 - 6.384	100	
Test of heterogeneity	Q=42.575, df=17, p<0.001; I^2=60.07% (95% CI 39.40-80.70)					

*PD: Panic disorder, OPD: Other psychiatric disorders, PA+: Number of participants experiencing panic attack following carbon dioxide challenge test, N: Number of total participants.

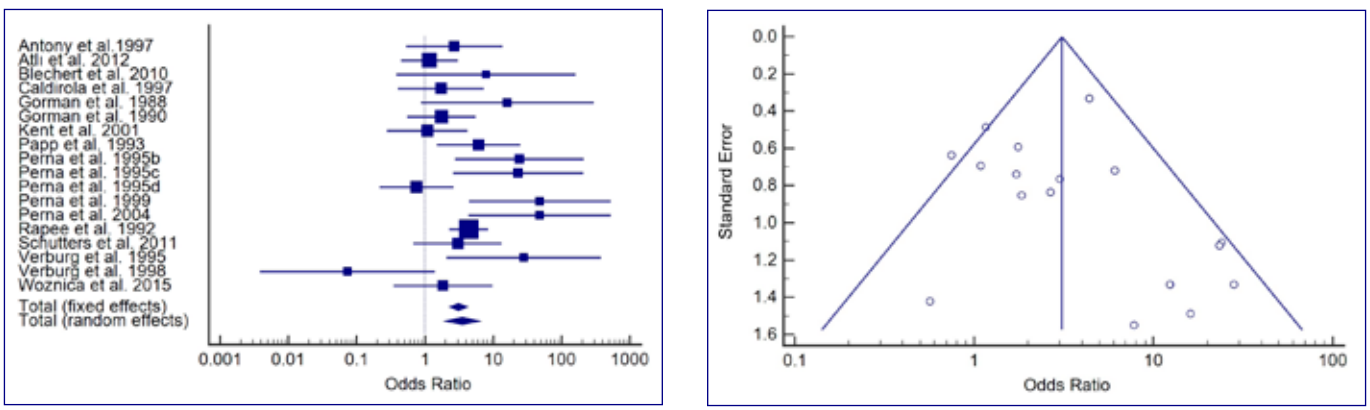

Figure 4. a) Forest Plot Graph of Odds Ratios From Studies Comparing PD and OPD Groups by Carbon Dioxide Inhalation Test, **B)** Funnel Plot for Publication Bias
Odds Ratio (random effects) = 3.524 (95%CI 1.945 - 6.384) in favor of PD

carbon dioxide inhalation test is approximately 4 according to the random effect model.

Comparison of Healthy Subjects with and without PD Diagnosed First-Degree Relatives

The respective data was available only in studies involving inhalation tests carried out with 35% carbon dioxide only, and this concentration was used in intergroup comparisons. After a 35% carbon dioxide inhalation test, the HC having first-degree relatives with PD were observed to experience approximately 3 times more PA than those without PD diagnosed first-degree relatives, and this difference was statistically significant (Table 5 and Figure 5a). Reviewing the data with respect to heterogeneity (I^2=17.28, p = 0.305) and

Table 5. Meta-analysis of of Studies Comparing PA Frequency Between Healthy Subjects Having First-Degree Relatives with PD with Those Having No First-Degree Relatives with PD

Study	Healthy subjects having first-degree relatives with PD (PA+/Total N)	Healthy subjects having no first-degree relatives with PD (PA+/Total N)	Odds Ratio	95% CI	Weight %	
					Fixed	Random
Coryell et al. 1997	5/11	0/15	26.231	1.259 - 546.463	1.025	2.906
Coryell et al. 2006	39/75	18/57	2.347	1.144 - 4.818	43.369	38.870
Perna et al. 1995a	5/23	1/44	11.944	1.302 - 109.587	2.373	7.292
Van Beek et al. 2000	21/50	12/50	2.293	0.972 - 5.410	30.744	29.984
Monkul et al. 2010	11/32	8/34	1.702	0.580 - 4.998	22.488	20.948
Total (fixed effects) (χ^2= 17.146, df=1, p<0.001)	81/191	39/200	2.658	1.678 - 4.212	100	
Total (random effects) (χ^2= 11.302, df=1, p<0.001)	81/191	39/200	2.591	1.487 - 4.514	100	
Test of heterogeneity	Q=4.836, df=4, p=0.305; I^2=17.28% (95% CI 0.00-69.90)					

*PD: Panic disorder, PA+: Number of participants experiencing panic attack following carbon dioxide challenge test, N: Number of total participants.

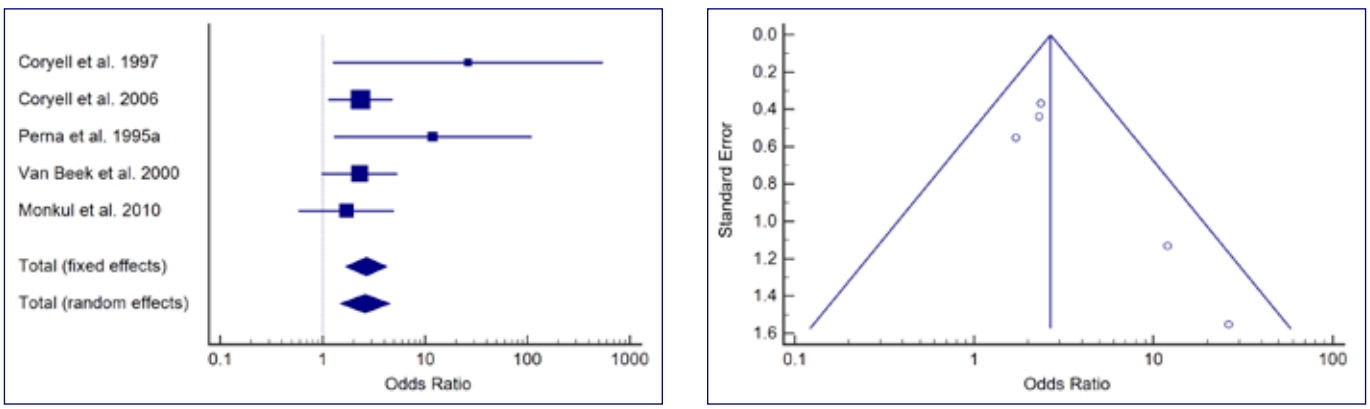

Figure 5. a) Forest Plot Graph of Odds Ratios From Studies Comparing Healthy Subjects Having First-Degree Relatives with PD with Those Having No First-Degree Relatives with PD by 35% Carbon Dioxide Inhalation Test, **B)** Funnel Plot gor Publication Bias

Odds Ratio (fixed effects) = 2.658 (95%CI 1.678 - 4.212) in favor of HC having first-degree relative with PD

publication bias (Begg-Mazumdar's: Kendall's tau = 0.4, p = 0.483; Egger's test = 1.97, 95% CI = -0.096 – 4.037, p = 0,056) did not indicate data that would adversely affect the analysis in a significant way (Figure 5b).

DISCUSSION

Increased sensitivity to carbon dioxide inhalation is the most important conceptual starting point of the biologic theories trying to explain and understand the etiology of PD. Increased sensitivity to carbon dioxide and high incidences of PA after a carbon dioxide inhalation test in subjects with PD has been supported by various clinical trials. However, comparisons of the odds ratios of having PA between people with PD and various mental disorders, and healthy individuals with and without first degree relatives with PD after inhaling different concentrations of carbon dioxide had not been clarified before the present meta-analysis. The results of our meta-analysis, the first in this field, clearly showed that there was an increased risk of PA induction after carbon dioxide inhalation for individuals with a first-degree family history of PD as well as those with PD diagnosis. Since the subjects with PD showed a significantly higher sensitivity to carbon dioxide inhalation as compared to subjects with OPD, the increased sensitivity to carbon dioxide inhalation may be considered to be specific to PD. A comparison between healthy subjects with and without first degree PD diagnosed relatives revealed that the former group had significantly higher sensitivity to carbon dioxide inhalation.Therefore, the sensitivity to carbon dioxide inhalation may be attributed to an inherited a PD susceptibility from parents even if the disorder (PD) does not emerge phenotypically (clinically) in the person.

The nature of the relationship between increased sensitivity to carbon dioxide inhalation and PD is not yet fully known. Nevertheless, the results of this meta-analysis support Klein's hypotheses on the etiology of PD (Klein 1993) according to which, physiologically emerging false suffocation alarms underlie unexpected PA. Impaired central carbon dioxide

regulating mechanisms underlie such unexpectedly emerging false suffocation alarms. Some peripheral indicators of such impaired central carbon dioxide regulating mechanisms are seen in patients with PD. These include frequent sighs and yawns, and the irregularities in the physiology of their respiratory system (Klein 1993).

The interaction between carbon dioxide and acid-sensing ion channels (ASIC) has been explored in relation to this phenomenon. Carbon dioxide is known to form an acidic solution when dissolved in water. There are reports on the presence of a polymorphism in the ASIC genes and a relationship of this polymorphism with the positive response to a carbon dioxide inhalation test (Savage et al. 2015, Smoller et al. 2014).

Although this meta-analysis clearly shows an increased sensitivity to carbon dioxide inhalation in individuals with PD and their healthy first-degree relatives as compared to healthy controls, it should be noted that there may be some known limitations to the study. Firstly, some study articles not presenting the data analyzed in this meta-analysis were therefore excluded from the study. Moreover, the results of the current study may have been influenced by the facts that the studies included in the meta-analysis used different methods for carbon dioxide inhalation tests, they did not present results specific to PD types accompanied or not accompanied by agoraphobia when presenting the study outcome, claustrophobia was not clearly mentioned as an inclusion or exclusion criterion and there were some differences in the definition of PA. A moderate heterogeneity was found when comparing the PD group to the OPD group. The most probable reason for this can be that many different mental disorders were assessed as if they belonged to the same group. Therefore, the real effect sizes may be less in the overall effect size (odds ratios) obtained, but no moderator analysis was attempted as it would have broadened the scope of this paper greatly.

Furthermore, if carbon dioxide inhalation definitely triggers panic attacks, a 35% carbon dioxide concentration would be expected to form more frequent panic attacks than a 5% carbon dioxide concentration. This study, however, did not find any clear difference of effect between the doses of carbon dioxide used in the inhalation tests. A possible explanation for the absence of a dose-response effect may be that the threshold is reached at low carbon dioxide concentrations (e.g. between 5-7%). Another reason for this observation may be the presence of just a few or no individuals in the healthy group who presented with panic attacks, resulting in low incidences which increased the error in the calculation of confidence interval for the odds ratios. The Peto Odds method could be used in this case, but since that method can also produce significant complications and biases, it has been recommended to be used as a last choice (Brockhause et al., 2016).

In conclusion, the results of this meta-analysis have supported the fact that the carbon dioxide inhalation test as a PA challenging method results in significantly increased PA frequency in individuals with PD. The measure of the effect size in the experimental interventions that generally have a categorical outcome is expressed as an odds ratio. When the odds ratios obtained here were approximated to the frequently used Cohen's d value, the effect size of carbon dioxide inhalation in provocation of PA was 1.492 for a 5% carbon dioxide concentration and 1.370 for a 35% concentration in subjects with PD; 0.694 in the OPD group, and 0.539 in the HC group having first degree relatives with PD, indicating a moderate or large effect size for all comparisons (Borenstein et al. 2009). The results obtained in this study support the fact that carbon dioxide inhalation strongly promotes panic attacks in patients with PD and their healthy first-degree relatives. Further studies may investigate in more detail the effect of carbon dioxide inhalation test as a challenge test on gender, agoraphobia and some panic attack subtypes.

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